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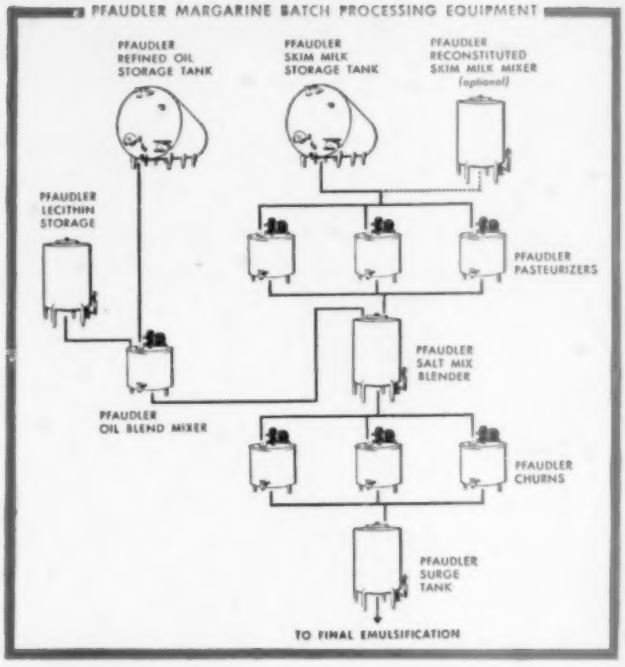

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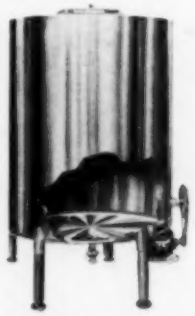

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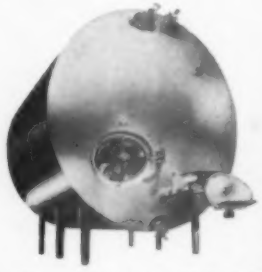

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Food Technology and World Health

The Preamble to the Constitution of the Food and Agriculture Organization of the United Nations provides that "The Nations accepting this Constitution, being determined to promote the common welfare by furthering separate and collective action on their part for the purposes of

> raising levels of nutrition and standards of living of the peoples under their respective jurisdictions,
>
> securing improvements in the efficiency of the production and distribution of all food and agricultural products,
>
> bettering the condition of rural populations,
>
> and thus contributing toward an expanding world economy,

hereby establish the Food and Agriculture Organization of the United Nations, hereinafter referred to as the 'Organization,' through which the Members will report to one another on the measures taken and the progress achieved in the fields of action set forth above."

Another organization, The World Health Organization, has given us one of the clearest definitions in the English language: "Health is the condition of physical, mental and social well-being and not the mere absence of disease or infirmity."

The objectives of both these world-wide organizations are so important to the future security of the world that we food technologists must consider searchingly what contribution we can make to implement them.

In examining our function as food technologists in the broadest meaning of this term:

1. It is obvious that we must give increasing attention to the proper storage conditions necessary to maintain our harvested food crops in pallatable condition with a maximum retention of their nutritive values. These considerations involve factors such as the moisture content of the food, its chemical nature, the presence or absence of air in the surrounding storage space, the size and physical construction of the storage unit, the possibilities of controlled composition and temperature of the storage air and other factors to meet local conditions.

2. Our raw food products at time of harvest can be preserved by such methods as dehydration, freezing, canning, dehydro-freezing, concentration, — the value and utility of which have been proved by time and use. Food technology, in cooperation with ancillary sciences such as chemistry, physics, biology, microbiology, and engineering can make substantial and worthwhile contribution to our world problem by the development of newer, cheaper, and even more effective methods of food preservation.

3. With our present concept of nutrition and the basic role which food plays, the food technologist and the horticulturist must visualize the possibilities of better foods and consequently better nutrition through the development of new varieties of food raw materials in which essential nutrients are concentrated to a higher degree than in existing varieties. We should not, however, close our minds to the possibilities of using inexpensive synthetic vitamins to improve the nutritive value of processed foods, especially where they form a major part of the consumer's diet.

4. Transportation of food raw materials and processed foods has always imposed problems of varying degrees of magnitude. Further studies of a technological character are certainly needed to give us desired answers to many of our transportational problems.

5. The elimination or at least the reduction in the amount of food waste along the entire chain from field to the ultimate consumer is another objective to which food technologists can make definite contributions. In the food processing plant where raw materials are assembled, the attention of technologists should be given to the utilization of waste material for either food or nonfood purposes. Fortunately, definite progress is being made in this field because of the economic and social considerations involved.

6. Education in the realm of human nutrition will enable the producer, processor and ultimate consumer to understand more clearly what is expected and desired. Demonstrations of good techniques especially in the production and preservation of raw materials will go a long way toward making more readily available the food which nature, under man's guidance and assistance, has produced.

There is no question in medical thinking about the relationship between proper food and well-being. A body optimally nourished, as to both the quality and the quantity of food intake, is certainly in a far better position to resist the inroads of disease than is the undernourished being.

The health records achieved in Great Britain during the period 1942-47 inclusive show clearly the advantages from improved food habits. Notwithstanding the many inconveniences and disarrangement of normal living habits, an intensive nutrition program produced amazing results. Material mortality (excluding abortion) declined by 46 percent, stillbirths declined by 28 percent, infant mortality declined by 13 percent, and neonatal deaths declined by 13 percent.

Even our thinking, which is so important in world affairs, will certainly be improved in clarity, soundness, and fraternity, if the mind is a part of a well-nourished body.

In 1944 Dr. Ancel Keys at the University of Minnesota began a large-scale experiment on the physiological and psychological changes in man following prolonged starvation. During the semi-starvation period of six months, the food intake was reduced to 1570 calories. At the end of this period, the average weight loss was 24 percent. In addition to the physical indications the strain produced increased emotional instability. As summarized in *Nutrition Reviews:*

> "An important feature of this study has far-reaching implications in the socio-political field. At the start of the experiment, the group was characterized by good humor, friendliness, and fellowship; democratic principles and spirit guided them in their actions. As the starvation period proceeded, the men lost some of their ability to make rational decisions. In three, possibly four men, finally dropped from the experiment, the moral fiber degraded to the point where some stealing of food occurred. Leadership and initiative among the men disappeared completely and they welcomed a firm direction. Starvation made them submissive. In the early phases of rehabilitation the prevailing mood was that of rebelliousness and hostility."

As the Latin saying goes "Mens sana in corpore sano"; so food technologists the world over can make a real contribution to world peace, health and happiness.

F. C. BLANCK,
President, I. F. T., '44-'45.

Prediction of Thiamine and Ascorbic Acid Stability in Stored Canned Foods[a]

MYER FREED, SADIE BRENNER, AND VIRGIL O. WODICKA[b]
QM Food and Container Institute for the Armed Forces, Food Research Division

The authors present nomographs which relate temperature, storage time and vitamin retention. These graphs permit prediction of retention values beyond the conditions of the actual experiments, by extrapolation. The results of other workers are compared favorably with the predicted values.

Introduction

Early studies in this laboratory have indicated that the loss of thiamine and ascorbic acid from miscellaneous food materials stored at constant temperature took the course of a first-order reaction *(7)*. Storage studies on representative canned foods used by the Army (apricots, orange juice, tomato juice, peas, spinach, green beans, and lima beans) were therefore conducted at three temperatures, 70°, 90°, 100° F. (21°, 32°, 38° C.) *(1)*, the minimum necessary to reveal if the classical Arrhenius equation was applicable. This was found to be the case. As a close approximation the logarithm of the retention and therefore the log of the storage life to a given retention were linearly related to the temperature (Figure 2). These simple relationships prompted an exploration of these storage data to determine if they could be used as a basis for (a) predicting stability of the two labile nutrients at storage temperatures and times outside the range originally studied, including variable temperatures; (b) estimating the limits of temperature and time at or below which a given retention (or better) of the nutrient in question may be obtained, and (c) predicting stability of these nutrients in foods similar to those employed in the original study.

This paper presents the temperature relationships found for the foods listed above in the form of nomographs. Their applicability to prediction are shown by comparing results of other workers at other temperatures with values predicted from these relationships.

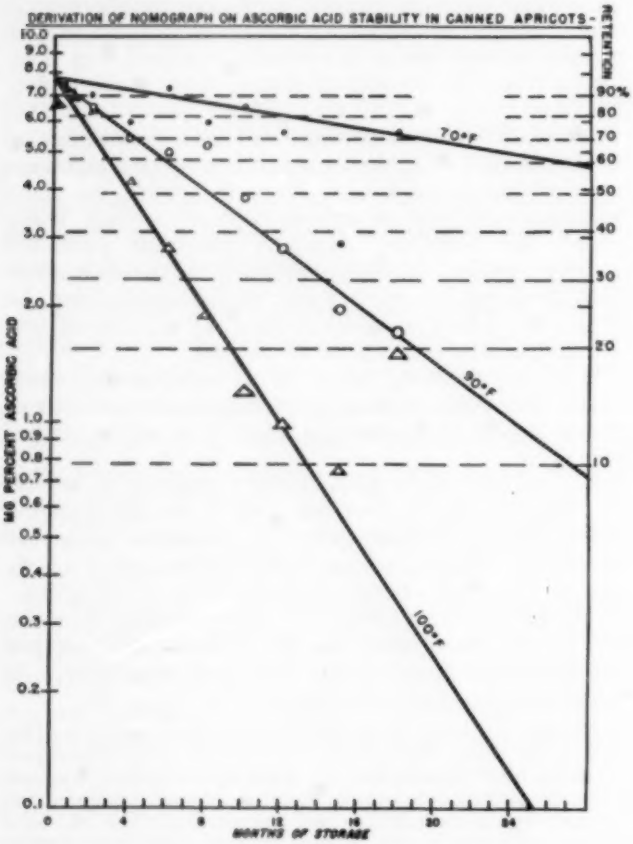

FIG. 1.
● — 70° F. ○ — 90° F. △ — 100° F.

Experimental Procedure

The experimentally determined vitamin values (mg. percent) were plotted on semi-logarithmic paper for each food against the months of storage for the three storage temperatures. The best possible straight lines

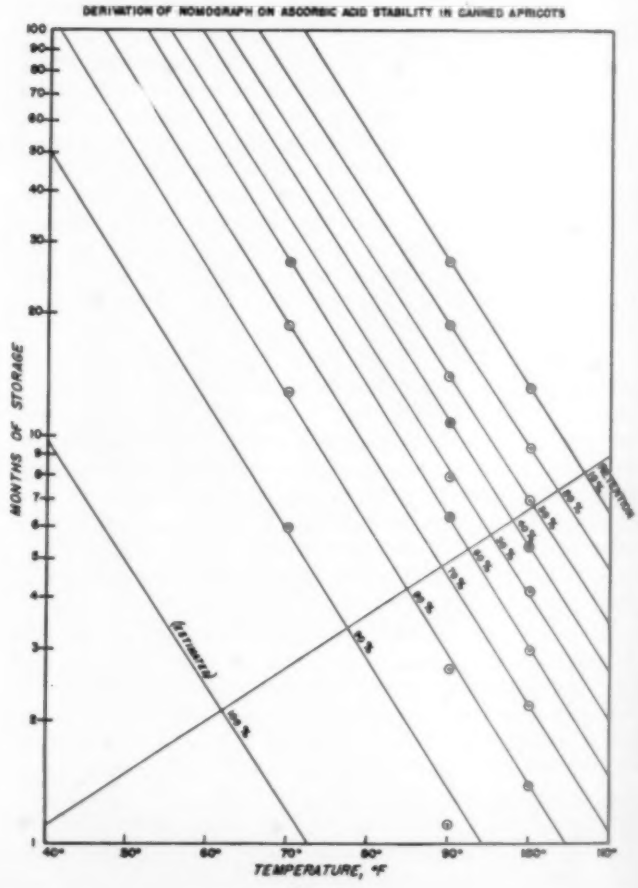

FIG. 2.

[a] This paper reports research undertaken by the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 221 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

[b] Libby, McNeill and Libby, Blue Island, Illinois.

representing the change in vitamin content during storage at each temperature were then drawn to meet at a common point on the mg. percent axis at zero months. This point was used as the initial vitamin value for each food.[c] Horizontal lines were then drawn across the graph at points which represented calculated retentions of 90% down to 10%, 10% intervals being employed. (See Figure 1). The intersections of these horizontal retention lines with the three constant temperature lines, enables one to read off the time in months which is required to bring about the given retention of the vitamin in question at the temperature indicated. To illustrate: the 80% retention line crosses the 70° F. (21° C.) line of ascorbic acid in apricots at 12.5 months, the 90° F. (32° C.) line at 2.7 months, and the 100° F. (38° C.) line at 1.4 months. Therefore, apricots retain 80% of their ascorbic acid for 12.5 months at 70° F., 2.7 months at 90° and 1.4 months at 100° F.

When log months were plotted against the temperature in degrees F. for each percent retention, a family of parallel straight lines was obtained. The family of parallel lines could be represented by their common perpendicular, it being necessary only to graduate the perpendicular to show where each line crossed. (Figure 2). Since the distances between these lines were logarithmically related it was possible to extend the graduations in both directions wherever necessary [d] by fitting a logarithmic scale to coincide with those graduations which had been experimentally determined. A nomograph [e] was then constructed from the data in Figure 2. This procedure was followed for both thiamine and ascorbic acid for each food studied by Brenner et al *(1)*. (See Figures 3 and 4).

Results and Discussion

To determine whether such nomographs can predict results obtained by other investigators for these foods stored at different temperatures, the data in Table 1 have been gathered from the literature and compared to values obtained from the nomograph. The results show good agreement between experimental and predicted values. It is obvious that these nomographs cannot be expected to give fully accurate results in the extremities of the temperature range if only because water freezes at 32° F. (0° C.) and browning increases rapidly at the higher temperatures. Furthermore, sam-

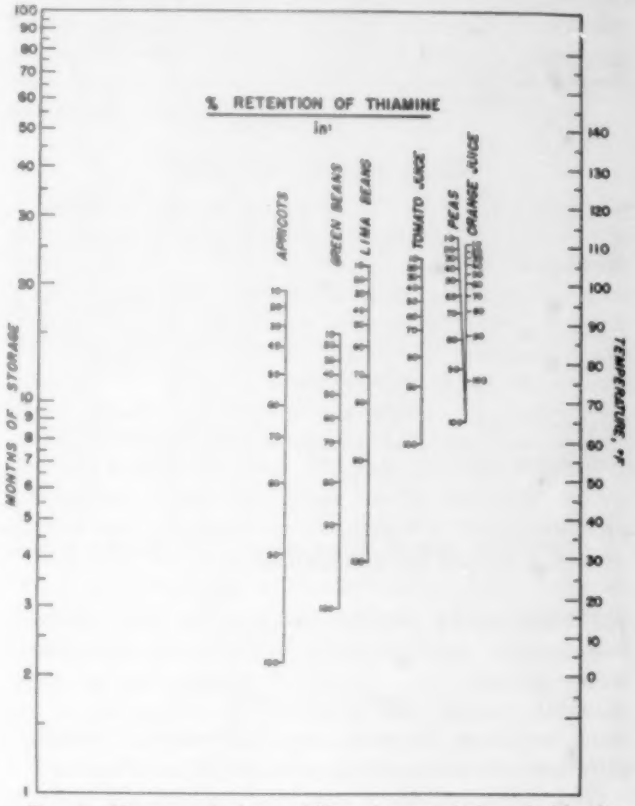

FIG. 3. Nomograph for stability characteristics of thiamine in canned foods.

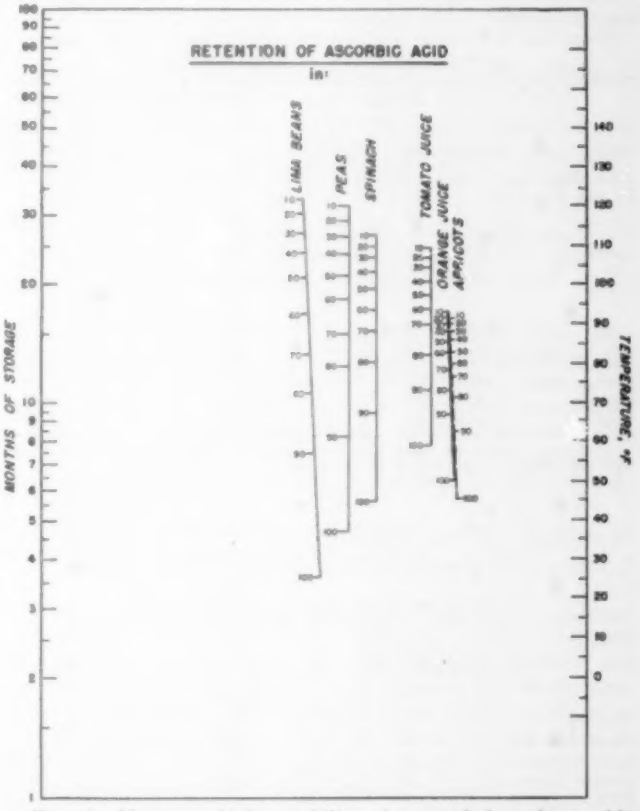

FIG. 4. Nomograph for stability characteristics of ascorbic acid in canned foods.

[c] This procedure was followed since the original analyses were just as much subject to error and sample variability as any others.

[d] The limited scale in Figure 1 (0-28 months) made it impractical to estimate the lower retention values directly for the more stable foods, and therefore, extrapolation was necessary in Figure 2. In every case 100% retention was determined in this way.

[e] The procedure for constructing the nomographs was as follows: A logarithmic (1 — 100 months) and a parallel linear scale 0 — 140° F. (— 18 — 60° C.) were marked off. Two well separated points were chosen from each constant retention line of Figure 2. Working lines were then drawn joining the readings on the scales representing the storage time and temperature of these two points for each constant retention. The intersection of these two lines represented the constant retention characteristic of the two points which were used.

For example, the intesection of the 100 months, 55° F. (13° C.) temperature working line with that of the 2.0 months, 110° F. (43.5° C.) temperature line represented the 50% constant retention point on the third scale being constructed. This same procedure was used to extend the scale at 10% intervals from 10 to 100% retention.

TABLE 1

Comparison of Predicted Storage Retention of Ascorbic Acid and Thiamine With Experimental Values Obtained by Various Workers

Product	Storage Temperature ° F.	Ascorbic Acid				Thiamine			
		6 mos. Stor.		12 mos. Stor.		6 mos. Stor.		12 mos. Stor.	
		Percent Ret. Found	Pred.	Found	Pred.	Found	Pred.	Found	Pred.
Tomato Juice (2)[f]	70	86	97	83	91	.....	.....	.....	.....
(3)[g]	30	100	100	92	100	100	100	92	100
	42	98	100	90	100	98	100	90	100
	85	90	90	74	79	89	89	71	79
	110	50	63	20	40	50	60	18	34
(6)[g]	50	92	100	100	98	100	100	95	100
	65	95	94	96	93	98	100	92	94
	80	98	93	86	84	92	92	84	84
Orange Juice (6)[g]	50	100	100	97	95	100	100	100	100
	65	96	97	92	90	98	100	98	100
	80	88	84	76	68	94	100	86	98
(5)	70	91	92	87	82	.....	.....	.....	.....
Peas (2)[f]	36	99	99	98	94	.....	.....	.....	.....
	70	87	90	84	83	.....	.....	.....	.....
	90	81	84	76	74	.....	.....	.....	.....
Spinach (2)[f]	70	82	84	76	83	.....	.....	.....	.....
Lima Beans (3)[g]	30	94	96	.....	.....	100	96	100	94
	42	84	94	.....	.....	96	94	96	91
	85	70	84	.....	.....	86	85	77	72
	110	60	74	.....	.....	41	57	22	32

[f] These values represent average retentions for food packed in glass containers and stored in the dark at the temperatures designated.
[g] Values estimated from published curves.

ple variation is considerable even in the relatively homogenous material from which these nomographs were derived *(1)*. Different experimenters, interseasonal, varietal and geographical differences in the food, variations in processing, etc., may all introduce differences of a completely uncontrollable character.

The data in Table 2 show the applicability of these nomographs for predicting storage stability under variable temperature conditions such as occurs in warehouse storage. It is clear from Table 2 that using yearly average temperatures, it is possible to predict within experimental error the vitamin retention for the foods enumerated. Mŏschette, Hinman, and Halliday *(6)*, Brenner, Wodicka, and Dunlop *(1)* have previously indicated this to be the case, by comparison of results of warehouse storage with data obtained at constant temperatures in the proximity of the average yearly temperature.

TABLE 2

Prediction of Ascorbic Acid and Thiamine Retention From Annual Average Temperature in Warehouse Storage

	Storage Temp.		Retention of Ascorbic Acid Percent		Retention of Thiamin Percent	
	Yearly Av. ° F.	Yearly Range ° F.	Found	Pred.	Found	Pred.
Orange Juice (6)	77	50- 98	73	72	96	98
	77	51- 91	81	72	95	98
	72	50- 92	81	79	99	100
	70	54-104	86	82	99	100
	66	51- 87	92	86	96	100
	63	42- 79	91	90	103	100
	61	36- 87	91	91	95	100
	59	28- 92	90	93	99	100
	58	30- 78	96	92	98	100
Orange Juice (1)[h]	77		75	72	72	98
Spinach (1)[h]	77		83	82	...	...
Peas (1)[h]	77		86	81	80	91

[h] Storage studies in same Florida warehouse used in *(6)*; average annual temperature assumed to be the same.

From these nomographs it was interesting to estimate the storage temperature at which a change in retention of ascorbic acid and thiamine would occur after one year's storage. Eighty percent retention at the end of this period was assumed by the authors to be a criterion of change. Table 3 illustrates the information obtained. It is observed that storage temperatures up to approximately 70° F. for periods of 1 year would not produce ascorbic acid losses exceeding 20% for orange juice, lima beans and apricots. In tomato juice, peas, and spinach, losses not exceeding 20% in 1 year could be obtained with storage temperatures up to 80° F. (27° C.). In the case of thiamine retention, storage temperatures up to 85° F. (29.5° C.) for tomato juice and peas and up to 95° F. (35° C.) for orange juice

TABLE 3

Predicted Vitamin Retention Characteristics of Canned Foods

	Ascorbic Acid				Thiamine			
	Max. Storage temp. for 12 mos. for 80% Retn.	Max. Storage time at 35° F. for 80% Retn.	Retn. for 12 mos. Storage at 35° F.	Retn. for 12 mos. Storage at 100° F.	Max. Storage temp. for 12 mos. for 80% Retn.	Max. Storage time at 35° F. for 80% Retn.	Retn. for 12 mos. Storage at 35° F.	Retn. for 12 mos. Storage at 100° F.
	° F.	Mos.	%	%	° F.	Mos.	%	%
Apricots	68	90	99	0	16	8	72	35
Green Beans	....	....	....	....	28	10	76	8
Lima Beans	67	25	90	63	64	33	92	48
Tomato Juice	84	100	100	63	83	100 +	100	60
Peas	79	45	93	68	88	100 +	100	68
Orange Juice	72	100	100	5	96	100 +	100	78
Spinach	81	62	97	68	....	.....	.....	....

resulted in losses not exceeding 20%. However, lower temperatures were needed to maintain the same thiamine stability in lima beans, apricots and green beans, namely 64°, 16°, and 28° F. (18°, —9°, and —2° C.) respectively.

Another interesting comparison was made on vitamin stability in the various foods at 35° F. (1.5° C.) to determine if the differences observed at the higher temperatures were maintained at the lower temperature. The number of months storage required at 35° F. to show a significant decline (80% retention) in nutrient content was estimated and the percent retention following 1 year's storage at 35° and 100° F. (1.5° and 38° C.) compared. It is indicated in Table 3 that the differences in stability of foods shown at the high temperature become less marked at the lower temperature as would be expected from the increased stability resulting at lower storage temperatures, (i.e. 0 and 5% ascorbic acid retention in apricots and orange juice respectively after 12 months' storage at 100° F., compared to the 65% retention for the other foods; 99 and 100% retention at 35° F. respectively compared to the 90-100% retention for the other foods). Another observation worthy of note is the apparent reversal of relative stability of ascorbic acid in peas and lima beans at the lower temperature. Although the maximum storage temperature to produce 80% retention of ascorbic acid in 1 year was practically identical for apricots and lima beans, and for orange juice and peas, the stability at 35° F. was almost four times as great for apricots as for lima beans and two times as great for orange juice as peas. In the peas and lima beans, retention of ascorbic acid did not show as marked a temperature relationship as shown for other foods. This may in part be explained by the starchy nature of these two foods and the subsequent formation of interfering substances which reacted as ascorbic acid on storage at elevated temperatures, thus producing apparently greater stability than in other foods.

The possibility of predicting retention of ascorbic acid and thiamine in foods of similar nature to those from which the nomographs were derived is indicated in Table 4. Retention for peaches, tomatoes and grapefruit juice was predicted from data based on apricots, tomato juice and orange juice respectively. It is seen that with the exception of thiamine retention in peaches,[1] satisfactory agreement of the found values with the predicted values was obtained. This comparison is an indication that the nomographs presented may be used for the purpose of predicting nutrient stability in similar foods. Caution should be used in employing these nomographs for such a purpose until more experimental data are available.

TABLE 4

Comparison of Predicted and Found Retention Values for Related Foods (6 months' storage)

	Storage Temperature ° F.	Ascorbic Acid		Thiamine	
		Found % Retn.	Predicted % Retn.	Found % Retn.	Predicted % Retn.
Peaches *(6)*	50	90	97	97	83
	65	84	91	97	74
	80	78	78	92	68
Tomatoes *(6)*	50	96	100	100	100
	65	92	99	95	99
	80	89	92	90	92
Grapefruit Jnice *(6)*	50	92	100	100	100
	65	90	93	98	100
	80	82	82	94	100
Grapefruit Juice *(5)*	70	94	92	....	....

Summary and Conclusions

Based on data obtained on several canned foods for 18 months' storage at three controlled storage temperatures, 70°, 90°, 100° F. (21°, 32°, and 38° C.), nomographs for ascorbic acid and thiamine retention for these foods were derived. These nomographs made it possible to extend the findings in terms of retention of the nutrients to constant and variable temperatures and times outside the range of the study. The type of information which may be obtained in terms of nutrient stability is demonstrated. Findings of other workers at temperatures other than those used to derive the nomographs were compared with predicted values from the nomograph and good agreement observed. The application of this technique to the prediction of the retention of nutrients in foods of like nature to those for which nomographs are given is also discussed.

Acknowledgment

Grateful acknowledgment is made to Dr. H. L. Fevold for his helpful suggestions and interest in the work.

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[1] Although peaches were considered a similar food to apricots, the initial thiamine concentration of peaches was approximately one-half that of apricots *(4)*, whereas the initial thiamine and ascorbic acid content of the other pairs of foods were of the same order of magnitude. This discrepancy may be the explanation for the disagreement in predicted and found thiamine values in peaches.

The Prooxidant Effect of Ascorbic Acid and Cysteine in Aqueous Fat Systems[a]

DOROTHY A. SCARBOROUGH[b] AND BETTY M. WATTS
College of Home Economics, Syracuse University, Syracuse, N. Y.

A new method for evaluating antioxidants and synergists in aqueous fat systems has been employed to demonstrate that aqueous solutions of either ascorbic acid or cysteine accelerate the oxidation of lard if no added primary inhibitor is present. The method has been used to study also the effect of other accelerators and inhibitors of rancidification.

The antioxidant effect of ascorbic acid suspended in vegetable fats *(4)* or lard containing added phenolic antioxidants *(2)* is well established. Its antioxidant activity has been shown to be dependent upon the presence of a primary inhibitor of the phenolic type. With lard containing no added antioxidant little protective effect was obtained in concentrations up to .2% *(4)*.

On the other hand, Krukovsky and coworkers have shown that ascorbic acid definitely accelerates the development of oxidized flavor in milk *(7, 8)*. The maximum acceleration took place when the ascorbic acid was present as a mixture of the reduced and dehydro forms. Rapid oxidation of ascorbic acid beyond the dehydro stage eliminated its prooxidant effect. While the exact relationship of oxidized flavor to unsaturated fat oxidation is somewhat obscure, it has been suggested that oxidation of phospholipids in the fat globule membranes may be the cause of the off flavor *(13)*.

Variable results were obtained in this laboratory when ascorbic acid in concentrations of .05% of the weight of meat was added to pork sausage preserved by freezing. In one experiment, the ascorbic acid had no effect on rancidity development in the fat. In two other experiments some retardation of rancidity was obtained, but more rapid dulling of the red color occurred. In the absence of ascorbic acid loss of red color correlated well with rancidity development *(16)*, but in the presence of added ascorbic acid, dulling of color was observed when the peroxide number of the extracted fat was zero.

In both foods (milk and meat) in which ascorbic acid produced deleterious effects, the ascorbic was in solution in an aqueous phase in contact with an animal fat having relatively small amounts of tocopherol. This study was therefore undertaken to investigate the effect of ascorbic acid and other synergistic antioxidants on rancidity in artificial systems containing an aqueous phase in contact with lard, both with and without added tocopherol. Experiments on hemoglobin and muscle extract were included in the investigation, since hemoglobin is known to catalyze both the oxidation of unsaturated fatty acids *(17)* and the oxidation of ascorbic acid *(1)*. The results should be of value in predicting the probable effect of these synergists in complex fatty foods containing an aqueous phase.

A second objective of the investigation was the establishment of a simple artificial system for testing antioxidants in an aqueous fat mixture. The method used in previous studies on the catalytic effect of muscle extracts *(9, 17)*, in which the aqueous solution was emulsified in the fat, is unsatisfactory in several respects. The amount of solution which can be incorporated in the fat is limited, the extent of contact between aqueous and fat phases is relatively small, and there are probably variations in degree of dispersion, depending on the emulsifying ability of the solutes in the aqueous phase.

Experimental Procedure

Aqueous fat system. The method finally adopted for obtaining contact between the water and fat was as follows: A 9 cm. filter paper (S & S No. 589, black ribbon) placed in a Petri dish absorbed evenly 1.5 ml. of the aqueous solution to be tested. Melted lard (1.5 gm.) containing .01% added carotene was weighed on to a second filter paper which was placed on top of the first. The fat penetrated rapidly throughout both papers, giving an even yellow color. (Various other filter papers tried either gave trouble from rippling and entrapped air or were too compact to allow penetration of the fat over the bottom paper.) Six drops of toluene were added to each dish as a preservative. The dishes were covered and placed on a rack in a tightly covered container with water in the bottom. When experiments lasted more than a few days the dishes were completely sealed with paraffin to prevent losses of moisture and preservative. The experiments were conducted in an air oven at 40° C., at which temperature the fat was completely melted.

Extraction and Analysis of fat. Bleaching of the carotene served as a visible indication of the early stages of fat oxidation. At the onset of rapid oxidation following the induction period any carotene present was completely bleached. In so far as possible the fat was extracted and peroxide determinations made at intervals after bleaching had progressed but before it was complete. Both papers were extracted with carbon tetrachloride. No drying agent was needed as the water was retained in the filter paper. The extract was filtered, an aliquot of the clear filtrate dried to determine the concentration of fat, and a second aliquot containing approximately 0.5 gram fat was taken for peroxide determinations *(15)*. The peroxide number was expressed as millimoles of peroxide per 1000 grams of fat.

The results were recorded in terms of hours required for a sample to reach a peroxide number of 20, considered to be the end of the induction period or the beginning of rancidity. The antioxidant index reported is the quotient of the induction period of the substrate

[a] Financed in part by a grant made by Swift and Co. to the Institute of Industrial Research, Syracuse University.

[b] Taken from the thesis submitted by Dorothy Scarborough in partial fulfillment of the requirements for the Degree of Master of Science. Present address: Division of Dairy Industry, University of California, Davis, California.

with antioxidant divided by the induction period of the substrate without antioxidant. Antioxidant indices below 1 indicate an accelerating effect on rancidity.

Hemoglobin, muscle extract and buffers. The preparation of hemoglobin and muscle extracts and determination of their hemoglobin concentrations have been previously described *(17)*. Since it was shown that the lipoxidase activity of muscle extract is largely dependent upon its pH, experiments were run in buffered solutions at pH values of 6.5 and 5.4 (representing high and low values of normal fresh pork), as well as in unbuffered solutions. A 0.1M borate buffer was used at pH 6.5 and both phosphate and acetate buffers (0.2M) at pH 5.4. Where added ascorbic acid, catalysts, etc. shifted the pH of the buffer, enough of the basic or acidic component of the solution was added to maintain the desired pH. The dilution of buffer at all pH values was 1 part of buffer to 2 parts of final solution, making the concentrations of phosphate and acetate 0.1M and of borate .05M. The borate and phosphate buffers were without effect on rancidity, whereas the acetate consistently accelerated fat oxidation (antioxidant index 0.6).

Preparation of fat substrate. Commercial brands of lard purchased at a local store were used. No samples were used which had an original peroxide number higher than 4. In most cases the peroxide number was zero. The carotene-lard mixture was prepared at the time of each experiment by combining 9 parts of pure lard with 1 part of a previously mixed carotene-lard concentrate. The concentrate was prepared from 1 part of crystalline carotene dissolved in chloroform to 1000 parts of lard. When tocopherol was used, the pure alpha-tocopherol was dissolved directly in the carotene-lard.

Results

Effect of Ascorbic Acid on Oxidation of Lard in Contact with Various Aqueous Solutions. In the absence of added phenolic antioxidants, ascorbic acid invariably accelerated the oxidation of lard. This was true both in the absence and presence of hemoglobin or muscle extract and in unbuffered as well as buffered systems. Fig 1 shows the results of a typical experiment in which ascorbic acid, hemoglobin and combinations of the two (unbuffered) were added to the same sample of lard. The curve representing the activity of combined hemoglobin and ascorbic acid was consistently steeper during the earlier stages of oxidation. This may indicate that the ascorbic acid is so rapidly oxidized in the presence of hemoglobin that its prooxidant activity is exerted only during the earlier stages of fat oxidation.

Table 1 summarizes all results obtained with ascorbic acid in the aqueous fat systems described. No differences were noted in the effect of ascorbic acid at pH 6.5 as compared with pH 5.4, or in its effect in acetate as compared with phosphate buffers at pH 5.4. The hemoglobin concentration in the preparations used ranged from .01 gram to .42 gram per 100 ml. of the aqueous solution brought in contact with the fat, but since no consistent differences were noted in the antioxidant index of ascorbic acid over this concentration range, all results with hemoglobin and muscle extract were grouped together, without regard to pH or hemoglobin concentration.

In the absence of added tocopherol, ascorbic acid had marked prooxidant activity in concentrations of .01% as well as 0.1%. In the presence of added tocopherol in concentrations ranging from .01 to .05% of the weight of fat, ascorbic acid showed slight antioxidant activity. The results were qualitatively the same with added hemoglobin or muscle extract, i. e. the ascorbic acid again acted as a prooxidant in the absence of added

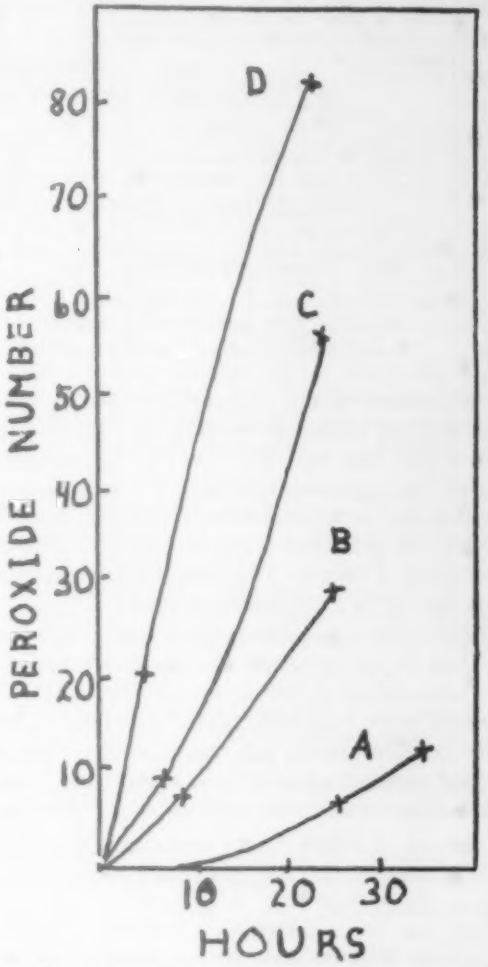

FIG. 1. Effect of hemoglobin and ascorbic acid on the oxidation of lard.
Curve A, water; Curve B, ascorbic acid solution (.1%); Curve C, hemoglobin solution (.42 gm. hemoglobin per 100 ml.); Curve D, hemoglobin (.42 gm., per 100 ml.)—Ascorbic acid solution (.1%).

TABLE 1

Effect of Aqueous Solutions of Ascorbic Acid on Rancidity in Lard

Added Tocopherol	Added Catalyst	Concentration of Ascorbic in % of Aqueous Solution	No. of Experiments	Antioxidant Index	
				Range	Average
None	None	.01	2	.2 — .3	.3
		.10	10	.06 — .7	.3
	Hemoglobin or Muscle Extract	.01	5	.7 — .9	.8
		.10	5	.4 — .8	.6
.01 to .05% of weight of fat	None	.10	5	.8 — 1.5	1.2
	Hemoglobin or Muscle Extract	.10	7	1.1 — 12.5	3.8

tocopherol and as an antioxidant in the presence of tocopherol. However, antioxidant indices obtained with ascorbic acid were regularly higher in the presence of hemoglobin or muscle extract. Possibly a preferential oxidation of ascorbic acid by the hemoglobin spares the fat.

Dry Fat as Compared to Aqueous Fat Systems. The prooxidant effect of ascorbic acid was not obtained in the absence of an aqueous phase. Table 2 gives the

TABLE 2

The Effect of Ascorbic Acid on Lard in Dry Fat As Compared With Aqueous Fat Systems

Experiment	Concentration of Ascorbic %	Buffer	Added Catalyst	Antioxidant Index	
				Aqueous-fat	Dry fat
1	.1	None	None	.06	1.2
2	.01	Acetate pH 5.4	None Muscle Ext.	.2 .8	.6 1.1
3	.01	Acetate pH 5.4	Muscle Ext.	.8	1.2
4	.01	Acetate pH 5.4	Hemoglobin	.8	1.0
5	.01	Borate pH 6.5	Hemoglobin	.7	.9

results of several direct comparisons of the effect of ascorbic acid on identical samples of lard in aqueous solution versus dry fat. For the dry fat experiments, 1.5 ml. of the aqueous solution (containing ascorbic acid, buffer and catalyst where the latter were used) were dried on the filter paper at 40° C. The drying required about 2 hours. The carotene-lard preparation was then run on to the dried filter paper.

In all cases the antioxidant indices were higher in the dry fat than in the presence of an aqueous phase. The average antioxidant index in the dry fat was 1.0. No experiments were run with added tocopherol, but the literature contains many references to the pronounced antioxidant effect of ascorbic acid in dry fats containing phenolic inhibitors in concentrations of .01% or greater.

Comparison of Filter Paper and Emulsion Methods. Table 3 illustrates the much greater effect of a pro-

TABLE 3

Comparison of the Prooxidant Effect of Muscle Extract in Emulsion and Filter Paper Systems

System	pH	Induction period in hours		Antioxidant Index
		Control .1 M acetate buffer	Added Muscle Extract (.04% hemoglobin)	
Emulsion	5.4	127	93	.7
Filter paper	5.4	115	15	.1

oxidant, in this case muscle extract, on oxidation of lard in the filter paper system described here as compared with the emulsion system previously employed *(17)*. The difference is undoubtedly to be ascribed to the greater area of contact between aqueous phase and fat in the filter paper system.

As might be expected, the prooxidant effect of ascorbic acid did not show up in the emulsion system. Antioxidant indices of 1.1 and 0.9 were obtained in two emulsion experiments with 0.1% ascorbic acid (no added hemoglobin or tocopherol) as compared to an average antioxidant index of 0.3 under the same conditions using the filter paper system.

Since it was considered possible that the prooxidant effect of ascorbic in the filter paper system might be due to the presence in the paper of traces of copper or other catalyst of ascorbic acid oxidation, the loss of reduced ascorbic acid was followed over a 24 hour period in the presence and absence of the filter paper. The amount of 0.1% ascorbic acid solution used in the fat oxidation experiments (1.5 ml.) was added either directly to an empty Petri dish or to a Petri dish containing an S & S 589 Black Ribbon filter paper. All dishes were placed in the oven at 40° C. The ascorbic acid was extracted with 0.4% oxalic acid at intervals and reduced ascorbic determined colorimetrically by a slight modification of the Loeffler and Ponting Method *(10)*. The ascorbic acid absorbed on the filter paper was oxidized at a slower rate than that added directly to the Petri dish. At the end of 5 hours 85% of the original ascorbic acid was recovered from the filter paper and 73% from the dish without paper. After 24 hours, 41% was recovered from the paper, 12% from the dish. The rate of oxidation might, of course, be different in the presence of the fat, but it seems evident that the filter paper does not accelerate ascorbic acid oxidation.

An experiment was also performed to eliminate the possibility that the results might be affected by solution of metallic catalysts from the glass. Filter papers impregnated with the aqueous solutions and fat were impaled on several short pieces of tooth pick imbedded in paraffin in the bottom of a Petri dish, thus eliminating all contact between the aqueous fat solution and the glass. Results obtained with papers suspended in this way are compared in Table 4 with papers similarly

TABLE 4

Effect of Contact With Glass on Rancidity Development in Lard

Aqueous Phase in Contact With Lard	Induction Period in Hours	
	Contact with Glass	No Contact with Glass
Water	108	115
Ascorbic acid solution (.1%)	7	7

treated but in contact with the glass dish. Little difference was obtained; the ascorbic acid solution was strongly prooxidant in both cases.

Cysteine and Other Synergists. The antioxidant effects of a number of amino acids, phosphates, etc. have been determined under the same conditions as the ascorbic acid. While a detailed report of the activity of these substances is not within the scope of this paper, no other synergist, with the exception of cysteine, gave antioxidant indices below 1.0. Cysteine, in 0.1% concentration, like ascorbic acid, was markedly prooxidant in aqueous fat systems containing no added tocopherol. Antioxidant indices of 0.3 and 0.4 were obtained in aqueous solutions of this amino acid in contact with carotene-lard. The corresponding values in the dry fat system were 0.7 and 0.9. Cysteine was not tried in the presence of added tocopherol, but its antioxidant

activity with fats containing phenolic inhibitors has been well established *(2)*. It is of interest that ascorbic acid and cysteine, the only synergists showing prooxidant activity in the aqueous fat system, are also the only ones readily and reversibly oxidizable.

Discussion

It is probable that the overall effect of ascorbic acid or cysteine will be antioxidant rather than prooxidant if conditions are such that the oxidation of the synergist is coupled with the reduction of the phenolic antioxidant. The coupled reaction might be expected to predominate when the concentration of phenolic inhibitor is high and when the synergists are distributed in the dry fat. On the other hand, when the synergists are dissolved in an aqueous solution in contact with a fat low in tocopherol, it is probable that oxidation of the synergist is largely independent of its reaction with the tocopherol.

Even when the overall effect of ascorbic acid on a fat system is that of an inhibitor, it may at some phase of the oxidation act as an accelerator. Filer et al *(3)* have noted that when large concentrations of ascorbic acid were added to vegetable fats, a rapid rise in peroxide value took place while amounts of ascorbic acid were still present, which if added directly, protected the fat. These authors suggested from their results that products of ascorbic acid oxidation might be prooxidant in nature.

If the prooxidant effect of ascorbic acid is dependent upon an equilibrium between the reduced and dehydro forms *(8)* many environmental factors might be expected to affect its activity, such as presence of catalysts of ascorbic acid oxidation, extent of contact between fat and aqueous phases and concentration of phenolic inhibitor. While partial oxidation of the ascorbic acid might cause it to accelerate rather than inhibit oxidation of fat, further oxidation of the ascorbic acid might be expected to eliminate its prooxidant effect. Before recommendations are made for its wide spread use in any complex food containing an aqueous phase, the antioxidant should be thoroughly tested under all conditions which might be encountered in the handling or storage of the food. Attempts to stabilize fats by feeding ascorbic acid to animals *(12)* seem of doubtful utility. In any event, more information is needed on the effect of the direct addition of ascorbic acid to meat and meat products.

If it were possible to incorporate primary antioxidants in the fatty tissues it is probable that ascorbic acid and other water soluble synergists could prolong the storage time of meat. While the tocopherol content of animal fats is dependent upon tocopherol in the diet *(5)*, relatively large amounts of diet tocopherol are required to bring about significant increases in resistance to rancidity of pork *(14)* or rabbit *(11)* fat. During the smoking of meat phenolic antioxidants are deposited *(6)*. Synergists might be expected to enhance the stabilization of the smoked product, but it is probable that synergists less open to objection than ascorbic acid and cysteine can be found.

Summary and Conclusions

1. An artificial system for testing antioxidants is described whereby extensive and uniform contact between an aqueous phase and fat is obtained.

2. Aqueous solutions of ascorbic acid or cysteine accelerate oxidation of lard containing no added primary inhibitors. The addition of ascorbic acid or cysteine to hemoglobin preparations causes further acceleration over that produced by the hemoglobin alone.

3. The accelerating effect of ascorbic acid is not obtained in the absence of the aqueous phase.

4. In the presence of .01% or more of added alpha tocopherol, ascorbic acid inhibits oxidation in aqueous fat systems.

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The Effect of Aging Versus Aging, Freezing and Thawing on the Palatability of Eviscerated Poultry[a]

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Effects of aging without freezing was compared with effects of aging, freezing and thawing on palatability of roasters and fowl. No difference was detected in aroma or flavor. There was a tendency for the birds frozen to be less juicy than those aged only. Tenderness was increased by both aging and freezing.

Introduction

Previous reports from this laboratory have shown that broilers undergo marked improvement in tenderness during the first few hours of aging following slaughter [Stewart, Lowe and Morr *(8)*; Hanson, Stewart and Lowe *(2)*]. If the carcass is cooked too soon after slaughter and before natural rigor sets in (e.g., within two hours), the meat is stringy, rubbery and tough; it is very difficult to chew. In contrast, if the carcass is allowed to age a few hours longer, the meat becomes tender. Breast muscles of broilers reach maximum tenderness within about 10 hours; thigh muscles tenderize in about 24 hours. Unpublished results from this laboratory have shown that similar changes in tenderness occur during the aging of roasters and fowl (hens). The time required for the development of tenderness in poultry is dependent upon the maturity of the stock, the older requiring the longest time; e.g., fowl (mature females) take about 48 hours to tenderize.

Aging is important from the standpoint of handling freshly-dressed poultry, especially in those instances where it is to be slaughtered, cooked and consumed within a comparatively short time. It seemed reasonable that aging might also be important in the handling of poultry to be frozen very shortly after slaughter. If so, proper aging of stock so handled would be important commercially, since it is becoming increasingly common to slaughter, dress, eviscerate, package and freeze poultry in one practically continuous operation. In many instances the total time required for handling the birds from slaughter to the onset of freezing is less than an hour.

A few studies have been made of this problem. Stewart et al. *(9)* reported no significant difference in tenderness between broilers frozen two hours after slaughter compared with those frozen 18 hours after slaughter. Interestingly enough, both lots of frozen birds were rated more tender than the fresh controls (unfrozen birds aged 24 hours prior to cooking). On the other hand, Wills et al. *(11)* found that fryers which had been frozen within two hours after slaughter were less tender than those aged 24 to 40 hours before freezing. Other studies indicate that freezing exerts a tenderizing effect on beef, the extent of which was dependent on aging time and freezing rate [Hankins and Hiner *(1)*; Hiner and Hankins *(3)*; Hiner, Maddsen and Hankins *(4)*; Tressler, Birdseye and Murray *(10)* and Paul and Child *(7)*].

The present experiments were designed to compare the effects of aging versus aging, freezing and thawing on the palatability of eviscerated poultry, particularly on tenderness.

Experimental

General Plan. The general scheme of the study follows: Birds were slaughtered, dressed and eviscerated. They were then aged for definite periods of time. The carcasses were next split in half. One of the halves was cooked at the end of the aging period and rated for palatability; the other half was frozen. After 24 hours the frozen half was removed from the freezer, thawed, cooked, and tested.

Except at the start and end of the experiment both frozen and unfrozen halves (but not from the same carcass) were cooked and rated at the same time.

The assignment of birds to a given aging time and a given freezing temperature was randomized.

Selection of Stock, Dressing and Evisceration. Fifteen Rhode Island Red and five Barred Plymouth Rock roasters (male birds 5 to 8 months old) and 20 White Leghorn fowl (female birds 20-24 months old) were used in this study. They were obtained from the College Poultry Farm where they had been raised under uniform conditions of environment and nutrition. The birds were slaughtered, bled, then semi-scalded by agitating vigorously for 1.5 minutes in two gallons of water adjusted to 58.9° C. (138° F.). The birds were then defeathered and promptly eviscerated. (Neck and feet were cut off; the neck skin was cut down the dorsal side, separated from the neck proper but left attached to the carcass skin. The neck was then cut from the carcass at the shoulders. Viscera were removed and discarded.) The birds were washed, then placed in Pliofilm bags and aged (0.5, 1, 2, 6 or 24 hours). Those held for two hours or less were kept at room temperature; those held longer were kept in a refrigerator at 4° C. (39.2° F.).

Freezing and Thawing. Thirty minutes before the end of the aging period (15 minutes for birds aged only 30 minutes) the birds were split in half along one side of the keel and through the back bone. Half birds (with and without keel bones) were randomized among the various treatments. In order to protect the muscles thus exposed, the neck skin was stretched over the body cavity and sewed to the skin of the breast and back. See Figure 1.

One-half of each bird was cooked and tested immediately after aging; the other half was frozen and thawed. Twenty halves were frozen at —17.8° C. (0° F.) and twenty at —34.4° C. (—30° F.). Freezing rate data were recorded automatically by means of a Leeds and

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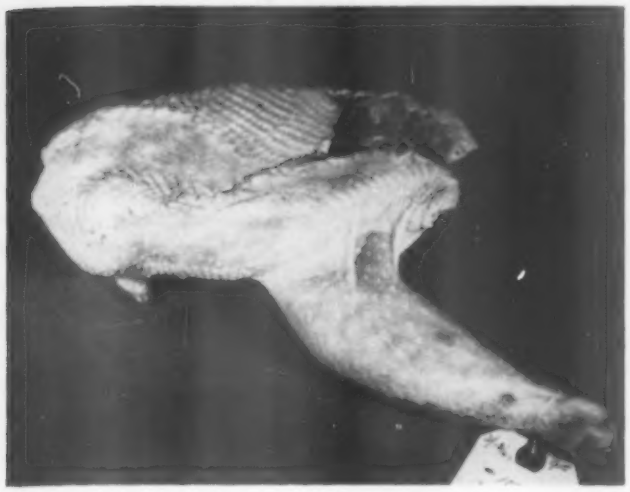

FIG. 1. Half poultry carcass showing method of protecting muscle exposed by cutting.

Northrup Micromaxcopper - constantan - thermocouple setup. (The thermocouple was placed in the center of the thickest portion of the thigh muscle.) These halves were left in the freezing rooms for 24 hours then removed and defrosted in a refrigerator for 24 hours at 4° C. (39.2° F.).

Palatability Tests. After aging or after aging, freezing and thawing, the halves were removed from the Pliofilm bags and examined visually for appearance and general condition. A short-stemmed thermometer was then inserted in the center of the thickest portion of the thigh muscle. The halves were roasted (breast side down on wire racks and placed in oval-shaped pans) in a gas oven at 150° C. (302° F.) until the thigh temperature reached 90.6° C. (195° F.).

The two breast muscles (pectoralis major and pectoralis secundus) were rated for palatability using subjective and objective tests. A panel of four experienced judges scored these muscles for aroma, flavor, tenderness and juiciness, using a scoring range of 0-10 (10 high). Each judge was given the same anatomical portion for scoring. Shear force tests for tenderness were made on the posterior portion of the pectoralis major remaining from the palatability tests. The apparatus used was the same as that described by Lowe and Stewart *(6)*.

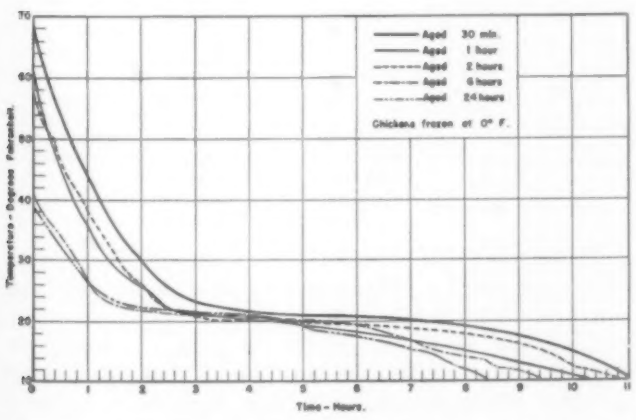

FIG. 2a. Rate of cooling and freezing of half chickens held at —17.8° C. (0° F.).

Results and Discussion

General. The average cooling and freezing curves are shown in Figures 2a and 2b. These data show that at —17.8° C. (0° F.) 8 and 9.5 hours, respectively, were required for freezing fowl and roasters and that 3 and 3.5 hours, respectively, were required at —34.4° C. (—30° F.). Whereas the freezing time between lots varied markedly, the difference is not as great as is encountered commercially.

The appearance of the carcasses when examined preparatory to cooking and scoring did not seem to be affected by the freezing and/or aging treatments.

Palatability. The objective and subjective palatability data are shown in Tables 1a ,1b, 2a and 2b.

Aroma scores varied only slightly (8.3 to 9.0). The differences obtained are not felt to be significant nor attributable to the induced variables.

Flavor exhibited a wide variability (6.9 to 9.5); however, the differences do not appear to be attributable to aging or freezing treatments.

Juiciness scores also varied widely. Juiciness appeared to decrease somewhat with aging before freezing. The data were not analyzed for significance, however. Of the 40 comparisons of freezing versus fresh, 28 showed the unfrozen halves to be the more juicy. In two instances, the scores for frozen and unfrozen halves were identical. In the remaining 10 cases the halves from the frozen group were more juicy. Although the significance of these differences was not tested statistically, the results indicate that freezing decreases the juiciness of poultry meat. Other data from this laboratory have shown statistically significant differences in the juiciness of fresh versus frozen muscle [Stewart, et al. *(9)*].

The results relative to the effects of treatment on tenderness are especially interesting. An analysis of

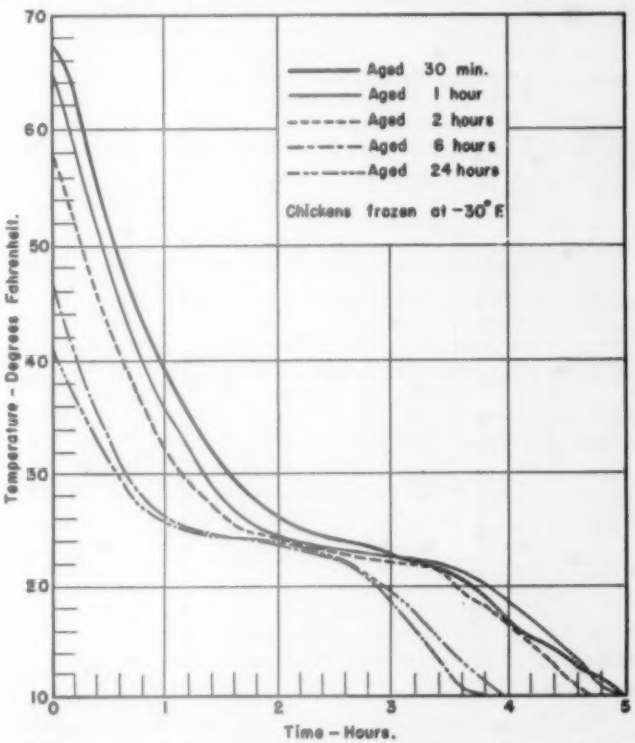

FIG. 2b. Rate of cooling and freezing of half chickens held at —34.4° C. (—30° F.).

TABLE 1a

The Effect of Aging and Aging, Freezing and Thawing on the Palatability of Roasters and Fowl (Frozen at —17.8° C.)

(Pectoralis Major)

Type	Treatment	Time of Aging Hrs.	Average Score*				Shear Force Lbs.
			Aroma	Flavor	Juiciness	Tenderness	
Roaster	Control	0.5	8.8	8.9	7.5	5.5	16.2
	Frozen	0.5	9.0	8.8	6.4	8.5	5.8
Fowl	Control	0.5	8.9	8.6	7.8	—6.5	30.8
	Frozen	0.5	9.0	8.6	6.6	7.6	7.4
Roaster	Control	1.0	9.0	9.5	6.0	2.8	15.9
	Frozen	1.0	9.0	8.8	5.6	6.3	6.8
Fowl	Control	1.0	8.8	7.5	5.6	—1.1	28.8
	Frozen	1.0	8.8	8.9	6.6	6.0	8.2
Roaster	Control	2.0	8.8	8.6	6.6	3.5	20.8
	Frozen	2.0	8.9	8.6	5.6	8.3	6.0
Fowl	Control	2.0	8.9	8.5	5.2	4.9	16.8
	Frozen	2.0	8.8	8.4	5.2	6.8	8.8
Roaster	Control	6.0	8.6	8.8	6.5	8.0	7.2
	Frozen	6.0	8.9	8.6	5.2	8.2	5.1
Fowl	Control	6.0	9.0	8.7	6.5	6.8	10.4
	Frozen	6.0	9.0	8.6	4.8	7.0	8.2
Roaster	Control	24.0	8.7	8.5	5.4	9.2	5.8
	Frozen	24.0	8.9	8.6	4.2	9.2	3.8
Fowl	Control	24.0	9.0	8.1	3.9	7.9	8.7
	Frozen	24.0	8.9	8.4	3.5	7.0	9.0

*Scoring range 0-10 (10 high) except for tenderness where scores ranged as low as —6. This extension of the range was necessitated by the extreme toughness of fresh fowl muscle.

TABLE 2a

The Effect of Aging and Aging, Freezing and Thawing on the Palatability of Roasters and Fowl (Frozen at —34.4° C.)

(Pectoralis Major)

Type	Treatment	Time of Aging Hrs.	Average Score*				Shear Force Lbs.
			Aroma	Flavor	Juiciness	Tenderness	
Roaster	Control	0.5	9.0	8.6	6.6	3.6	17.8
	Frozen	0.5	9.0	8.8	6.3	8.5	5.4
Fowl	Control	0.5	8.9	8.4	7.3	2.0	27.8
	Frozen	0.5	9.0	8.5	4.5	8.3	7.4
Roaster	Control	1.0	8.4	7.9	6.3	2.4	22.8
	Frozen	1.0	9.0	8.7	6.7	8.8	4.1
Fowl	Control	1.0	8.9	8.4	5.6	—0.6	28.8
	Frozen	1.0	8.9	7.9	6.7	6.0	8.2
Roaster	Control	2.0	8.6	8.2	5.8	2.0	20.1
	Frozen	2.0	9.0	8.6	5.6	8.1	5.6
Fowl	Control	2.0	8.6	8.0	6.5	—0.4	27.0
	Frozen	2.0	8.9	8.8	6.4	8.0	10.1
Roaster	Control	6.0	8.8	8.6	5.8	9.2	7.6
	Frozen	6.0	9.0	9.0	6.2	7.9	7.0
Fowl	Control	6.0	8.3	8.3	6.3	2.6	18.3
	Frozen	6.0	8.9	8.4	5.8	6.6	8.2
Roaster	Control	24.0	8.8	8.4	5.0	9.3	5.4
	Frozen	24.0	8.8	8.8	5.6	9.4	5.2
Fowl	Control	24.0	8.4	8.4	6.6	8.3	5.5
	Frozen	24.0	8.8	8.4	5.4	6.4	6.7

*See Table 1a footnote.

variance of these data was made. Figure 3 illustrates some of the effects of freezing and/or aging on tenderness.

The data confirm earlier results in that the poultry carcasses showed marked improvement in tenderness as a result of aging.

TABLE 1b

The Effect of Aging and Aging, Freezing and Thawing on the Palatability of Roasters and Fowl (Frozen at —17.8° C.)

(Pectoralis Secundus)

Type	Treatment	Time of Aging Hrs.	Average Score*			
			Aroma	Flavor	Juiciness	Tenderness
Roaster	Control	0.5	8.9	8.8	6.0	7.4
	Frozen	0.5	8.9	8.8	4.4	8.9
Fowl	Control	0.5	8.9	8.7	6.4	—2.3
	Frozen	0.5	9.0	8.8	4.6	7.6
Roaster	Control	1.0	9.0	8.6	3.9	7.5
	Frozen	1.0	9.0	8.4	3.9	8.9
Fowl	Control	1.0	8.9	6.9	4.6	2.5
	Frozen	1.0	8.8	8.0	4.4	5.8
Roaster	Control	2.0	8.8	8.8	4.4	7.4
	Frozen	2.0	8.9	7.9	3.9	7.3
Fowl	Control	2.0	8.8	8.3	5.4	4.9
	Frozen	2.0	8.8	8.1	4.2	5.6
Roaster	Control	6.0	8.5	8.5	4.9	9.1
	Frozen	6.0	9.0	8.5	3.0	6.4
Fowl	Control	6.0	9.0	8.4	5.9	6.6
	Frozen	6.0	9.0	8.5	3.0	6.4
Roaster	Control	24.0	8.6	7.9	5.2	9.2
	Frozen	24.0	8.6	8.4	3.6	8.8
Fowl	Control	24.0	8.9	8.4	2.6	7.4
	Frozen	24.0	8.8	8.2	3.2	8.3

*See Table 1a footnote.

TABLE 2b

The Effect of Aging and Aging, Freezing and Thawing on the Palatability of Roasters and Fowl (Frozen at —34.4° C.)

(Pectoralis Secundus)

Type	Treatment	Time of Aging Hrs.	Average Score*			
			Aroma	Flavor	Juiciness	Tenderness
Roaster	Control	0.5	9.0	8.5	4.6	5.2
	Frozen	0.5	9.0	8.4	4.2	9.2
Fowl	Control	0.5	8.8	8.4	4.4	4.6
	Frozen	0.5	9.0	8.4	3.9	6.5
Roaster	Control	1.0	8.5	7.6	4.6	4.6
	Frozen	1.0	8.8	8.0	4.9	9.1
Fowl	Control	1.0	8.9	7.6	4.6	2.5
	Frozen	1.0	9.0	7.2	4.4	5.8
Roaster	Control	2.0	8.6	8.2	4.9	4.2
	Frozen	2.0	8.9	7.0	4.2	8.8
Fowl	Control	2.0	8.6	8.3	4.5	5.2
	Frozen	2.0	8.9	7.8	5.0	5.4
Roaster	Control	6.0	8.8	8.2	4.2	8.8
	Frozen	6.0	9.0	8.4	4.5	8.8
Fowl	Control	6.0	8.6	7.9	5.2	6.5
	Frozen	6.0	8.9	8.4	4.6	7.9
Roaster	Control	24.0	8.8	7.8	2.5	8.5
	Frozen	24.0	8.8	8.4	4.6	7.3
Fowl	Control	24.0	8.8	8.2	4.6	8.8
	Frozen	24.0	8.8	7.6	3.8	7.6

*See Table 1a footnote.

The tabular data (Tables 1a, 1b, 2a, and 2b) appear to be anomalous in that tenderness first decreased, then increased. This result is thought to be due to the fact that too few birds (two for each group) were tested at each aging period. The combining of the data from the two different freezing temperatures and the two different types of poultry for Figure 3 eliminates the anomaly. Unpublished data involving a larger number

of birds have shown that tenderness increases roughly linearly with aging time (up to 24-48 hours).

Freezing significantly increased the tenderness of both roasters and fowl provided the carcasses had been aged six hours or less. Those aged 24 hours were not significantly affected by freezing. Of course the latter carcasses were already tender; apparently freezing did not increase the tenderness beyond what was accomplished by aging.

Fowl were never as tender as roasters. This has been observed repeatedly and is common knowledge to persons familiar with poultry meat. When the first palatability tests were made on fowl, it was found that those aged for very short periods of time were so extremely tough compared to what the judges were accustomed to as to require an extension of the scoring range. This accounts for the use of negative scores.

Shear force data paralleled the results of subjective appraisal of tenderness in all cases.

There was no significant difference in the palatability data between halves frozen at 0° F. and those frozen at —30° F.

Attention is drawn to the fact that in this study the effect of freezing *per se* has not been separated from the effect of thawing. It should be recalled that the frozen birds were thawed for 24 hours prior to cooking. A considerable amount of tenderizing may have resulted from the relatively slow defrosting. A study on the effects of slow versus rapid thawing is underway.

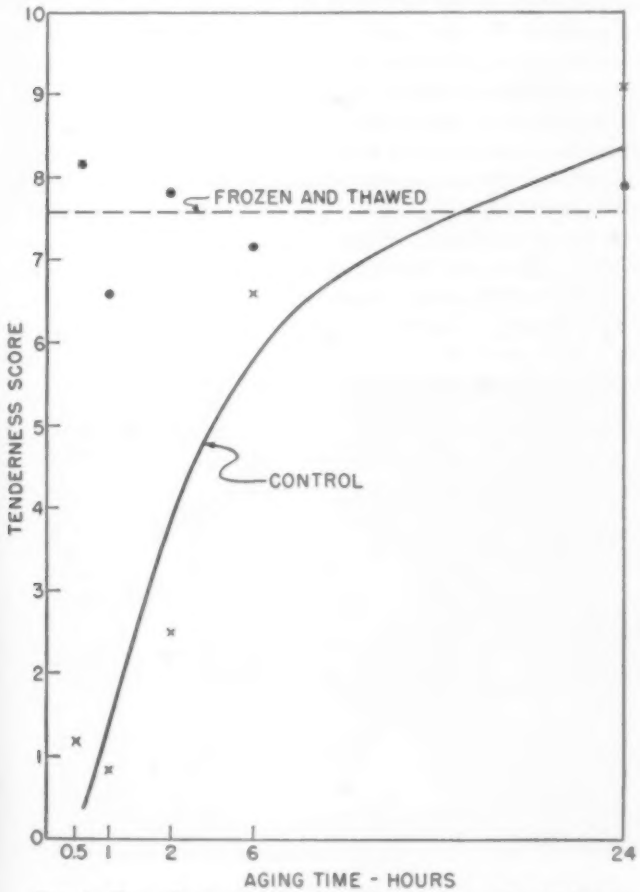

FIG. 3. The effect of freezing and/or aging on the tenderness of breast muscle (pectoralis major).
(The data for the two different freezing temperatures and two types of birds were combined.)

Summary

Twenty eviscerated roasters and 20 eviscerated fowl were aged for varying periods, then split in two. Forty of the halves were then frozen (20 at 0° F. and 20 at —30° F.). After aging, or after aging, freezing and thawing, the halves were cooked and rated for palatability using subjective and objective criteria.

No differences in aroma or flavor were detected which could be attributed to the various treatments used.

Birds which had been aged and frozen showed a tendency to be less juicy than those that had been aged only. Aging appeared to decrease juiciness somewhat also.

The tenderness of the birds was markedly affected by treatment. The unfrozen birds tenderized rapidly; high scores were recorded for birds aged 24 hours. Freezing increased tenderness markedly in those cases where halves had been aged less than six hours. In those cases where they had been aged 24 hours, there was little difference between frozen and unfrozen halves.

The effects of freezing on tenderness observed in this study may be confounded with the effects of thawing since the half carcasses were defrosted rather slowly.

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Acknowledgment

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Keeping Quality of Frozen Bakery Products

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Experiments were conducted on the freezing of bakery products both before and after baking. It was found that a larger variety of items can be frozen satisfactorily after baking than before; however, none of them were as good as when fresh from the oven. When unbaked items that lend themselves to freezing are baked in the home, they have much of the character of home prepared products.

The innovation of merchandising bakery products in frozen form has attracted considerable attention. No information is available on the total volume of frozen baked goods sold in the few years since they have been on the market; but, judging from articles that have been published describing freezing operations and distribution methods, significant quantities covering a wide variety of items have been produced.

The products frozen may be divided into two classes: (1) those that are baked and need only to be defrosted before use, and (2) those that are partially or fully prepared for baking but must be baked before they can be served. Commercially, the second class has received the most attention. In examining each of these classes we see that they fall into two categories: yeast-raised items that require proofing (fermentation time) before baking and items that contain no yeast and require no proofing.

Examination of a substantial number of commercially frozen bakery items now on the market has shown that, for the most part, they were not packaged to prevent loss of moisture over any prolonged period of frozen storage. Generally the package consisted of an ordinary pastry box and possibly an over-wrap having very poor moisture-vapor-proof characteristics. Such inadequate wrapping may have been used because the producers did not anticipate a long frozen storage period for their products, but were using freezing primarily as a novel sales approach. The labels of some products carried the statement that they should be used within 60 days. In the long run the expectation of such short life seems unsatisfactory, both to producer and consumer. The producer who is interested in frozen bakery products as a means of using slack production time or for the utilization of seasonal ingredients when they are in abundance should certainly expect to keep his products from 6 months to a year. Consumers, especially those who have home freezers or rent lockers, have come to expect frozen products to maintain satisfactory quality for many months, and are accustomed to season-to-season operation on fruits, vegetables and meats. Users of freezers also tend to keep frozen products much longer than they think they will when the materials are preserved.

Experimental

To secure information on the effect of prolonged storage on the quality of frozen bakery products, storage tests were made on both baked and unbaked items. All products were made according to commercial formulas. The baked items were wrapped as soon as they were cool and then frozen immediately. The unbaked items were frozen as son as they were made and wrapped after freezing. The freezing was done in a plate freezer at —25° F. in still air, and the products were stored in a walk-in box at —15° F. All of the products were packaged in pastry boxes and given a sealed overwrap of a cellophane having a good rating in regard to the prevention of moisture-vapor transmission under the conditions of freezing storage. The smaller items were sealed in cellophane individually or in small lots before they were packaged in the pastry boxes, so most of the items were double wrapped with moisture-proof material. All of the fruits used, with the exception of the peaches which were canned freestones, were commercially frozen. Table 1 lists the items included in the test and gives the time and temperatures at which the frozen unbaked items were baked.

TABLE 1

Frozen Bakery Products Examined

	Temp. of oven ° F.	Time of bake minutes	Frozen Before Baking Quality at:			Frozen After Baking Quality at:		
			6 mo.	9 mo.	14 mo.	6 mo.	9 mo.	14 mo.
Butterfly buns	390	15	U	U	....	S	S	....
Peach braid coffee cake	390	25	U	U	....	S	S	....
Baking powder biscuits	380	19	U	U	....	S	S	....
Apple squares	400	50	S	S	....	S	S	....
French cheese cake	410	45	U	U	....	S	S	....
Christmas tree cookies	380	10	S	S	S	S	S	S
Cocoanut molasses cookies	380	10	S	S	S	S	S	S
Small bridge cookies	380	20	S	S	S	S	S	S
Almond macaroons	380	20	S	S	S	S	S	S
Chocolate chip cookies	380	15	S	S	S	S	S	S
Mince pie	450	35	U	U	....	U	U	....
Mince turnovers	450	20	U	U	....	U	U	....
Apple turnovers	450	20	S	S	....	S	S	....
Cherry pie	450	40	S	S	....	U	U	....
Fruit and nut ring	....	....	....	....	....	S	S	....
Cherry tarts	....	....	....	....	....	S	S	....
Father's Day decorated cake	....	....	....	....	....	S	S	S
Baked Alaska	....	....	....	....	....	....	S	S

S—Satisfactory.
U—Unsatisfactory.

In order to evaluate the effect of different types of flours and varying amounts of shortening upon the quality of pie crust, cherry pies were made with crusts of differing compositions. Four flours were used:

1. First grade bleached cake flour.
2. First grade unbleached cake flour.
3. Unbleached pie flour.
4. Bleached pie flour.

The amount of shortening was the same for all flours: 50% based on the flour weight. In another series where the amount of shortening was varied, unbleached pie flour was used. Crusts were made having 25, 37½, 50, 62½ and 75% vegetable shortening, based on flour weight.

The frozen products were examined after 6 and 9 months' storage, and some at 14 months. The items frozen before baking that required no proofing were placed in the oven without defrosting. The yeast-raised items that required a proofing period before baking were defrosted, given a proof of 2 to 2½ hours at 90° F., and then placed in the oven. The proofing period was necessarily long because of decreased yeast activity. Corresponding items that had been frozen after baking were thawed at 75°-80° F. while still wrapped, the thawing being timed so that the items would be ready for tasting when the items that had to be baked were cool.

Results: Quality of the Products

Items baked before freezing. Most of the products that were frozen after baking (see Table 1) were found to have good quality after both 6 and 9 months storage. The Father's Day cake was in good condition after 14 months storage, and no rancidity had developed in the cream frosting. After 14 months the meringue of the Baked Alaska had lost some moisture and had become somewhat chewy, but the ice cream was in excellent condition. Although the products that were considered satisfactory did not have all of the fine eating quality of freshly baked products, they were salable and edible.

The mince pies and the cherry pies were the only items that were unsatisfactory. The mince pie developed an unpleasant flavor, and the top of the filling was covered with a white incrustation of unidentified composition. The gel structure of the cherry pie was broken down, giving a watery pie having poor eating quality.

Items baked after freezing. All of the yeast-raised items, the mince pie, baking powder biscuits, and the French cheese cake were found to have poor quality after 6 months. The yeast-raised items did not proof well, had poor volume, were heavy and soggy, and had poor crumb color. They were decidedly unappetizing in taste and appearance, and could not be eaten with pleasure. The baking powder biscuits had an unpleasant taste, were smaller in volume than those that were baked before freezing, and the crumb color was dull and had a yellow-grey tinge. Their external appearance was satisfactory. The French cheese cake had a soggy bottom crust, the volume was less than that of the frozen baked, and the cheese filling had an unpleasant flavor. The shortcomings of the mince pie were the same as those of the frozen baked.

All of the other items were judged to be good to excellent. The apple squares were covered with crumbs which tended to break away from the surface during freezing and handling. By use of care when the frozen squares were baked, the loose crumbs could be returned to their proper place and were well anchored when the bake was finished.

The four different flours gave no outstanding differences in regard to soaking of the crust or in the baking of those that were frozen before baking. Varying the amount of shortening did influence the quality of the finished product. The lean crusts, containing 25 and 37½% of shortening, respectively, baked out well but had poor eating quality. The 50% crust was the most satisfactory, as it baked well, showed little soaking, and was rich enough to give a firm, flaky crust having good eating quality.

Discussion

With the exception of the items noted above, all of the items that were baked and then frozen had satisfactory eating quality upon defrosting. They were not

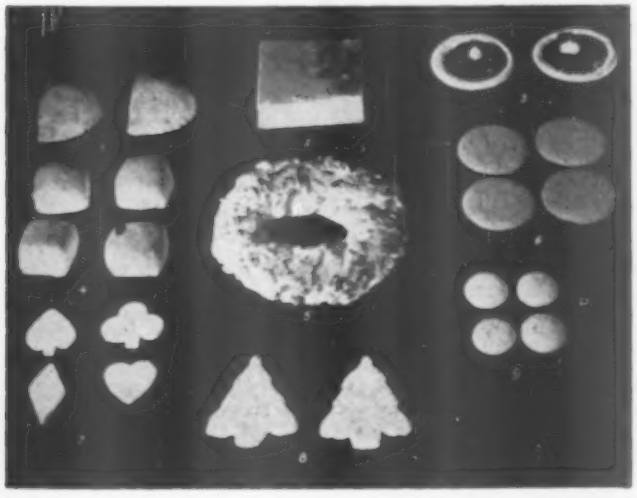

FIG. 1. Some of the items used in the work are shown: 1. Apple Turnovers; 2. French Cheese Cake; 3. Cherry Tarts; 4. Baking Powder Biscuits; 5. Fruit and Nut Ring; 6. Cocoanut Molasses Cookies; 7. Small Bridge Cookies; 8. Christmas Tree Cookies; 9. Almond Macaroons.

FIG. 2. Father's Day cake used in the experiments. The icing is butter cream type.

as good as if they were just out of the oven, but were probably equal in quality to baked goods one or two days old. An exception should be made in the case of cookies. They were rated as good as those a few hours old. The length of time over which items can be kept in frozen storage satisfactorily is influenced by the temperature of storage, the uniformity of temperature, and the moisture-vapor proof characteristics of the package. An even temperature of 0° F. or below is essential, but loss of moisture must be avoided under all conditions.

Table 1 shows that some items frozen prior to baking did not maintain satisfactory quality for even the six month period. In general a larger variety of items can be satisfactorily preserved by freezing after baking than by freezing before baking. This is especially true of yeast-raised items. The unsatisfactory results obtained with yeast-raised items before baking were thought to be due to the weakening or killing of the yeast during storage. This surmise was confirmed by experiments conducted by other workers in this laboratory. The complete results of their investigations have been published in another report *(1)*.

Baked items that are properly wrapped and frozen are principally advantageous to the housewife in being ready for use, provided adequate storage for frozen products is available in the household refrigerator or in a freezer.

With the frozen unbaked items that freeze satisfactorily, it is possible to achieve the quality and character of home-prepared goods. The same pleasant aroma results when frozen and freshly prepared goods are baked, an aroma that is not present in the kitchen except when baking is actually done there. This is the chief advantage of frozen unbaked products. They may be served as something that has been made with arduous toil and special skill by the housewife.

A second advantage of freezing before baking is that it makes it possible for the housewife to bake items in the home that she would not normally have the ingredients to make or the skill and tools essential for their proper production. Such items as Christmas Tree cookies and party cookies take special cutters and ingredients that the housewife does not normally keep on hand. By using the frozen unbaked cookies that are already cut and decorated, the homemade character can be achieved without the purchase of additional materials and equipment. It is in the specialty items that the greatest success may be expected.

Although frozen unbaked pastries relieve the housewife from the work of preparation, their baking must be given considerable attention if satisfactory results are to be achieved. Instructions for baking must be followed with care. It may be that the baking temperatures given are not the best for a particular oven; if satisfactory results are not obtained, other temperatures and different placing of the items in the oven should be tried. Pies may bake unevenly, the edge of the upper crust being overbaked when the center is not done. A protecting paper or cloth band placed on the edge of the pie will help overcome this difficulty. Whatever frozen products are being baked, the housewife should exercise as much care as she would in baking items made up in her own kitchen.

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Operation of a Routine Testing Group in a Small Laboratory

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Procedures used in the routine testing of foods for flavor, color and texture are described. Panel methods are used in which six to ten selected persons score the product in question. Products are also scored using paired and triple comparison procedures with larger groups.

It is generally agreed that there are, at present, no adequate chemical methods of evaluating the flavor of food resulting from various methods of preparation, processing and conditions of storage. Since fine flavor is a major goal of all of the work of the development of products and determines their acceptability, as well as commercial value, considerable effort should be expended in the development of better methods for the organoleptic examination of food-stuffs. Experiences during World War II certainly demonstrated the need of flavor and acceptability studies of foods prior to their incorporation in rations for the armed forces.

Members of the Division of Food Science and Technology of the New York State Agricultural Experiment Station at Geneva, feel very strongly the need of adequate flavor testing methods for processed foods.

This paper is a brief report of flavor-testing techniques used at the present time in Geneva. Some of the newer methods are used and the type of product, number of qualified people and amount of product available for flavor testing influence the procedure employed.

The Department has a wide variety of products for flavor comparison such as canned and frozen vegetables, frozen fruits, fruit juices, fruit ices, maple sirup, and jellies and jams. It is evident that we could not hope to have sufficient personnel with experience in scoring such a variety of products. It is necessary to select flavor tasters and to gain experience as we work with the various products.

Panel methods are used in which six to ten selected persons score the product in question. Products are also scored using paired and triple comparison procedures with larger groups.

A numerical scale of 1 to 10 in which an increase in

quality parallels an increase in numerical value is being used for all products. A sample rated excellent is given a score of 10; good falls in the range of 9, 8, 7; fair, 6, 5; poor, 4, 3, and very poor, 2, 1.

The score card has spaces allotted for the product scored, name of person, date, and comments. Many types of such scales varying in one or more respects are used in the scoring of products, other scales may be better than this one, but the use of such a scale gives uniformity in scoring products and is helpful in analyzing the results.

As a matter of illustration, one of our problems was to make flavor, texture, and color comparisons of canned peas, corn, wax beans, lima beans, and spinach which had been electronically blanched in comparison with commercial types of blanching. It was necessary to use a panel of eight persons in the scoring of these products since there was not a sufficient supply of samples for other procedures. We graded the canned peas first, and prior to comparing the experimental samples, we made comparisons of commercial samples of grade A. B, and C canned peas. We used a triple comparison procedure in which two samples were identical and one sample different. This procedure helped us in gaining experience in the scoring of peas and in the selection of personnel for the panel. In this test, the panel scored at one time six samples of experimentally blanched peas along with commercial samples of grade A and grade B canned peas.

In this type of panel scoring, wherever possible, especially with persons lacking experience in the scoring of a product, it is desirable to select a very good sample, a fair sample and a poor sample of the product for reference standards. These samples should be scored by the panel and the averages of the scores for each sample will designate the score of the sample. These samples can then be used as reference samples in scoring the experimental samples.

The scores of the panel members were averaged and their deviation from the average determined. When it is desirable to determine the significance of the difference between two mean scores, Fisher's *(1)* statistical formula is applied. We found that the electronically blanched peas had an off-flavor which did not occur in the water blanched peas. A number of the panel members felt that it was a bitter flavor.

As another illustration, members of the Department were studying physical and chemical changes of commercial samples of strawberry preserves, raspberry jam and grape jelly resulting from different times and temperatures of storage. Flavor changes were included in this study for comparisons with the other changes. Paired and triple comparison procedures were used. For example, in one test with strawberry preserves, each person making the comparison was given two samples of the control stored at 34° F. (1.1° C.), and one sample of the strawberry preserves stored at 100° F. (37.8° C.) for 14 weeks. Each sample was designated by a series of letters or a series of a combination of numbers and letters, as a code. In this way, persons working next to each other in a room would have the same sample with different code numbers, which would discourage a comparison with their neighbor. Only one set of two or three samples was served at one time. When the color of samples changed, due to time and temperature of storage, the room was darkened to cover up the differences in color.

As a matter of interest, I can briefly give the results of the flavor scores of the test on strawberry preserves. Fifteen members of the Department scored the samples and they did not know that in the series of three samples, two were identical. In the test comparing the two control samples stored at 34° F. (1.1° C.) for 14 weeks, with the sample stored at 100° F. (37.8° C.) for 14 weeks, nine of the 15 persons gave identical scores to the control samples and six persons gave a score of one point difference in the samples. As it happened, the average score of both controls was 8.9 and the sample stored for 14 weeks at 100° F. (37.8° C.) had an average score of 5.2. As indicated by the scores, there was a 100-percent preference for the control sample over that for the stored sample. The sample stored at 100° F. (37.8° C.) had darkened in color and had a somewhat cooked flavor, but there were no objectionable off-flavors. In comparing the control with two samples stored 14 weeks at 60° F. (15.6° C.), no significant difference was found in the average scores of the samples. However, three persons gave the identical samples stored at 60° F. (15.6° C.) for 14 weeks a difference of three points in score, and one a difference of four points in score. We knew that their flavor judgment was either off for that day or that they were guessing. These scores were eliminated from the averages.

The use of the score card for the paired and triple comparison procedures is helpful in analyzing the results. In recent reports on flavor testing the triple comparison procedure is highly recommended and we have found it to be very satisfactory when it can be used. We, likewise, found that eight or nine of the tasters who gave the most consistent results in the triple comparison procedure gave identical results in the panel method of scoring the same product.

In summary, I might say that we use panel methods in which six to ten selected persons score the product in question and products are also scored using paired and triple comparison procedures with larger groups.

We work in a room free from odors and disturbances and feel that it is desirable to have the members of the panel relaxed and comfortable. Needless to say all of the samples are scored "blind," that is, no member of the panel knows the identity of the samples.

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Effect of Freezing Rate on Fruits[a]

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Very slow, very rapid, and intermediate rates of freezing did not result in marked differences in vitamin content, appearance, flavor or texture and in their retention during a six-month storage period at 0°F. for strawberries, raspberries, and sliced peaches.

Many conflicting statements found in the literature relative to the effect of the rate of freezing on the quality of vegetables were pointed out in an earlier paper by the authors *(7)*. A review of the literature disclosed the same lack of uniformity of opinion concerning the effect of the rate of freezing on the quality of fruits. The following are illustrative.

Woodroof *(19)* stated: "A short interval between the formation of the first ice crystals and complete hardening of the product is essential for the preservation of texture, palatability and superficial appearance of fruits and vegetables. Heat removal should be sufficiently rapid to cause ice formation to progress in tissue at the rate of three-tenths centimeter per minute, at which rate a medium size strawberry will freeze in five minutes and harden in twenty-five minutes."

Lutz, Caldwell, and Moon *(9)* found: "Lack of agreement with the results of Woodroof *(20, 22)* as to the microscopic appearance of the rapidly frozen material may be due to differences in rate of freezing or in the details of handling the material. . . . The results reported herein do not indicate that rapid freezing has any marked advantages over slow freezing in the preservation of peaches, strawberries, and apple cider by the frozen pack method." These authors found more darkening in thawed fast frozen than in the thawed slow frozen peaches *(2, 9)*. They further stated (referring to peaches) "In no case was the rapidly frozen product made from any variety superior to that of the same variety which was slow frozen."

Joslyn and Marsh *(6)* stated: "It has been our experience that with the possible exception of asparagus, increasing the rate of freezing by using solid carbon dioxide does not appreciably improve the texture of fruits and vegetables examined."

Overholser and others *(14)* stated: "Experience has shown, however, that factors other than rate of freezing largely determine the quality of a pack."

Wiegand *(18)* (referring to berries) stated: "It seemed, however, from the results obtained, that the pre-treatment given the fruit was the most important factor to be considered. After the proper pre-treatment any good freezing method will prove satisfactory and will give a uniformly well colored and flavored product."

Morris and Barker *(12)* wrote concerning strawberries: "Quick freezing by immersion in brine at —28° C. or in liquid air, and subsequent storage at —10° C. or —20° C. offered no advantage over ordinary freezing." And concerning raspberries: " . . . after three months' storage the product was almost equally good whether quickly frozen in liquid air and subsequently stored at —20 or —10° C. or whether frozen at —20 or —10° C. either in air or in syrup." These same authors (again referring to strawberries) later *(13)* wrote: "After storage in cartons at —20° C. for four months, the samples frozen by direct immersion were superior to those frozen more slowly (i.e., in tins immersed in —20° C. brine or in cartons in air at —20° C.). Slow freezing in air at —5° C. gave the poorest products."

Birdseye *(1)* reported: "Slow-freezing results in very large ice crystals and considerably greater tissue damage, leakage, and change of appearance. With many of the more delicate fruits and vegetables, slow-freezing results in a distant change of flavor. . . . In extremely quickly frozen products on the other hand, tissue damage, oxidation, fermentation, and organic growth are reduced to a minimum."

Heiss *(4, 5)* found that fruits packed with sugar could be satisfactorily frozen slowly, provided that the start of fermentation is avoided. Heiss also made observations on ascorbic acid in connection with the rate of freezing of strawberries and some other fruits.

MacArthur *(10)* in a paper on asparagus, strawberries, and corn stated: "It was established that when freezing was by the slower methods the tissues were badly torn by internal ice crystals, while the more rapid methods caused less damage." No experimental evidence was given to support this statement.

Diehl *(3)* in 1932 made an excellent summary of the early work on freezing, with many references.

The present study is a continuation of work published by the authors on the effect of the rate of freezing on vegetables *(7, 8)*. It was carried on to determine whether extreme differences in the rate of freezing of strawberries, raspberries, and peaches would show noticeable differences in texture, color, flavor, or vitamin content.

Varieties and Experimental Packaging

Strawberries of the Dresden variety were harvested so as to obtain material as nearly uniformly ripe as possible. The berries were washed in cold water, capped, and sliced. Equal quantities of sliced berries, accurately weighed, were placed in each box. Then they were covered with weights of 60 percent cane sugar sirup that were the same for each box. This was done so that an entire box could be used for the ascorbic acid determinations, thus putting the results on a comparable fresh basis without further calculations. Sirup rather than sugar was used in strawberries to avoid physical damage to the slices during packing.

Raspberries, Newburgh variety, were packed whole and covered with 50 percent cane sugar sirup. Equal

[a] Approved by the Director of the New York State Agri. Experiment Station, Geneva, New York. Journal Paper No. 777, September 20, 1948.

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quantities of the berries, accurately weighed, were packed into each box. As described under strawberries, the berries in each package were covered with equal weights of sirup.

Elberta peaches were peeled with steam and sliced, and equally weighed amounts packed into cartons. Again, each carton was covered with equal weights of 50 percent cane sugar sirup, containing added ascorbic acid to retard the development of the brown color on thawing, as recommended by Tressler and DuBois *(17)*. Because of an error during preparation of this sirup, considerably less ascorbic acid was used than is normally recommended for this purpose.

Waxed cardboard pint-sized Vapocans[c] measuring 2⅞ inches high by 3¼ inches by 3¼ inches were used for the products described in this paper, except those frozen in liquid air. For these, Lily-Tulip[d] tub-shaped cartons were used. After they were frozen, but before they were placed in the 0° F. storage, the cartons were quickly and separately wrapped in moisture-vapor-proof cellophane. This step is unnecessary under ordinary conditions, but it was taken to make doubly sure that no moisture would be lost and thus the several samples would be strictly comparable.

Freezing Procedure

The same equipment and procedure for freezing were used as described in the previous paper *(8)*.

The most rapid freezing was obtained by immersing the uncovered cartons of fruit and sirup in liquid air. Other lots of samples were frozen at —40° F. in a blast of air and CO_2 in an Aminco cabinet. The blast was obtained from a fan supported so that the moving gas was directed at the packages from above, which provided a uniform velocity (650 l.f.p.m.) on all the boxes. Preliminary runs showed that all boxes froze in substantially the same time. Another lot was frozen in still air at 0° F. A third lot was put into the 0° F. unit, but the cartons were in the corrugated insulated boxes described in the previous paper *(8)*. Temperature changes in the boxes were determined by means of thermocouples connected with a Brown electronic recording potentiometer.

The freezing records produced by the potentiometer are shown in Table 1. The freezing zone was taken as 32° F. to 21.2° F. *(15)*.

Some difference in the times required to freeze the several products were noted. No attempt was made to freeze them under exactly the same conditions. We were interested in obtaining different freezing rates for a given product, rather than making any comparison of the time required to freeze different fruits under comparable conditions.

Preparation of Photomicrographs

The preparation of the sections of the frozen products was carried out in a refrigerated room at 0° F. with precautions being taken to prevent even partial thawing during sectioning. The sections for photographing were prepared by making a cut with a cold new razor blade through the frozen block of fruit or berries in sugar sirup so as to obtain a smooth section. A cube approximately one inch on a side was thus prepared for photographing. For raspberries and strawberries one or more seeds were included in the smooth cut sections to facilitate focusing and to orient the observer. For peaches, since no distinct tissues or sharp cell demarcation were evident, a sharp edge of the cut block was included in the field to facilitate focusing.

For comparison with the samples in the frozen state, separate sections of the products were prepared as described and then allowed to thaw at room temperature. Because the peaches and berries did not retain a smooth cross section after thawing, perhaps in part owing to the presence of the sirup, and because no distinct tissue changes associated with freezing rate were evident even in the frozen sections, no photomicrographs were made of peaches, raspberries, or strawberries after thawing.

The photomicrographs were made with a 35 mm. Leica copying camera on a usual type of panchromatic film. Lighting was obtained from a Leitz spotlight directed at the specimen from a relatively low angle. A low power specimen stage was used for focusing; the stage could be raised or lowered with the specimen, and thus the bellows distance was kept intact and similar magnification for a given series was assured. The pictures were made, therefore, by reflected light.

Analytical Methods

Entire cartons were used for the determination of ascorbic acid, as described earlier in this paper. The peach samples were ground in a food-chopper twice in the 0° F. room, followed by careful mixing to ensure uniformity. These were used for carotene determinations.

[c] Container Corporation of America, Chicago, Ill.
[d] Lily-Tulip Cup Corporation, New York, N. Y.

TABLE 1

Recorded Freezing Rates for Strawberries, Raspberries, and Peaches

Freezing Condition	Location of Thermocouples	Strawberries		Raspberries		Peaches	
		Time in Freezing Range	Time from Starting Temp. to 0° F.	Time in Freezing Range	Time from Starting Temp. to 0° F.	Time in Freezing Range	Time from Starting Temp. to 0° F.
Air Blast (Not insulated) —40° F. (Ambient)	Near outer side	35 m.	2 h., 40 m.	15 m.	35 m.	25 m.	2 h., 0 m.
	Center	1 h., 50 m.	3 h., 40 m.	50 m.	2 h., 5 m.	1 h., 30 m.	2 h., 55 m.
Still Air (Not Insulated) 0° F. (Ambient)	Near outer side	3 h., 5 m.	14 h., 10 m.	3 h., 20 m.	8 h., 40 m.	5 h., 10 m.	14 h., 0 m.
	Center	5 h., 14 m.	15 h., 30 m.	4 h., 30 m.	8 h., 40 m.	6 h., 58 m.	15 h., 30 m.
Still Air (Insulated Box) 0° F. (Ambient)	Near outer part	31 h., 0 m.	74 h., 40 m.	14 h., 0 m.	34 h., 0 m.	39 h., 10 m.	97 h., 50 m.
	Center (as described)	35 h., 5 m.	74 h., 40 m.	18 h., 49 m.	34 h., 20 m.	46 h., 15 m.	97 h., 50 m.

Ascorbic acid was determined by the indophenol-xylene extraction method *(16)*, and carotene by a diphasic method including a chromatographing step. This latter includes an extraction by a modification of the method of Moore and Ely *(11)* using a 3:1 mixture of ethanol and petroleum ether. The filtered extract was washed with water, with 25 percent of KOH in 80 percent ethanol, and again with water until neutral to litmus paper. The pigments were chromatographed on a mixture (1:1) of adsorptive powdered magnesia No. 2641 [e] and Filtercell [f], eluted with petroleum ether containing 0.1 percent of ethyl alcohol (Dr. G. H. Ellis, personal communication) and finally read in a spectrophotometer at 450 mμ.

Samples of the raw material were taken for analysis before the freezing was started. After the freezing was completed, a set of sample was taken to the laboratory for immediate analysis.

The rest of the frozen packages were held in storage at 0° F. for six months to determine whether any quality changes took place during this period. After the elapse of this period of time, a second set of each lot was analyzed.

Results

Palatability. The thawed samples were examined for color, texture, and flavor by a group of six qualified judges who were uninformed concerning the treatments until the tests were concluded. No differences could be detected among the samples regardless of the rate of freezing except in the case of the peaches frozen in liquid air. This sample had an off flavor, but as far as texture and color were concerned no differences could be detected. It is well to remember that these products were sirup packed, as in most commercial plants, and results should not therefore be compared with products obtained by freezing and packing of individual units. It is unlikely, however, that the vitamin picture would be different after such freezing.

We do not wish to create the impression that the use of the slowest freezing conditions described in this paper is recommended. Under such conditions, fermentation might conceivably get started, which would be undesirable. These very slow rates were included in this study for comparative purposes only. Also, rate of freezing should not be confused with overall time of handling. This work deals only with a comparison of the results of the several rates of freezing described.

Tissue Sections. The photomicrographs of the frozen samples (Figures 1-10) did not show any appreciable variation for the several rates of freezing of strawberries and raspberries. The results for peaches were similar. It appears, therefore, that the texture of these products when packed in sirup is relatively unaffected by the rate of freezing. Pictures of the cut sections of commercially frozen products are shown for comparison.

Vitamin Content. Data on carotene in peaches, and ascorbic acid in strawberries, raspberries, and peaches

FIG. 1. Strawberries frozen in liquid air and stored for six months at 0° F.

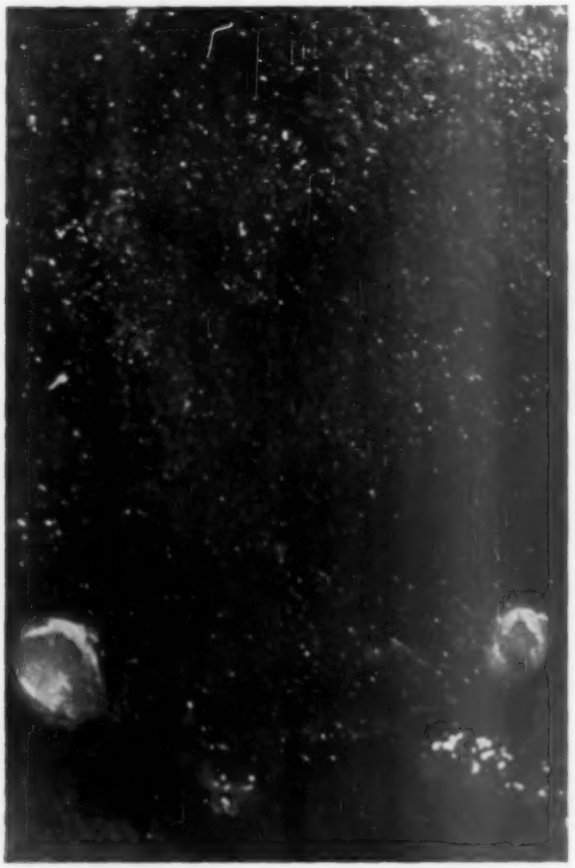

FIG. 2. Strawberries frozen with an air blast at —40° F. and stored for six months at 0° F.

[e] Westvaco Chlorine Products Corporation, Newark, California.

[f] Johns-Manville Co.

FIG. 3. Strawberries frozen in still air at 0° F. and stored for six months at 0° F.

FIG. 4. Strawberries frozen in an insulated box in still air at 0° F. and stored for six months at 0° F.

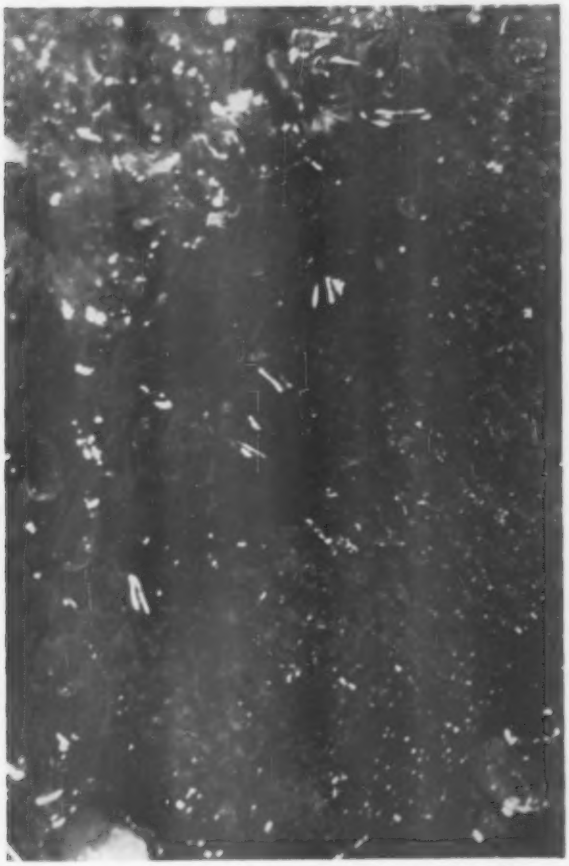

FIG. 5. Commercially frozen strawberries bought on the market.

FIG. 6. Raspberries frozen in liquid air and stored for six months at 0° F.

FIG. 7. Raspberries with an air blast at —40° F. and stored for six months at 0° F.

FIG. 8. Raspberries frozen in still air at 0° F. and stored for six months at 0° F.

FIG. 9. Raspberries frozen in an insulated box in still air at 0° F. and stored for six months at 0° F.

FIG. 10. Commercially frozen raspberries bought on the market.

TABLE 2

Analyses of Strawberries, Raspberries, and Peaches After Different Speeds of Freezing (Wet Basis)
The results are corrected for the weight of sirup added, hence are comparable.
1946

Sample	Strawberries		Raspberries		Peaches			
	Ascorbic acid Mg./Gm.		Ascorbic acid Mg./Gm.		Ascorbic acid Mg./Gm.		Carotene Mcg./Gm.	
	A	B	A	B	A	B	A	B
Raw	0.83	.....	0.38	.....	0.09	.....	14.2	.....
Liquid air	0.71	0.62	0.35	0.31	0.15*	0.13*	13.0	15.5
—40° F. with air blast	0.70	0.64	0.35	0.36	0.14*	0.14*	13.9	15.8
0° F. not insulated still air	0.75	0.72	0.36	0.32	0.13*	0.13*	14.4	14.6
0° F. insulated still air	0.63	0.56	0.34	0.33	0.12*	0.12*	13.0	16.7

A = Raw and immediately after freezing.
B = Analyzed after 6 months' storage.
* = Included added ascorbic acid to prevent browning.

(Table 2) show that even with the very slow rate of freezing there were only insignificant differences in vitamin content. After six months' storage at 0° F., similar results were obtained, indicating that the stability during storage was not impaired. It seems evident that the freezing rate did not affect the nutritive value of the fruit as measured by these vitamins.

Summary

Strawberries, raspberries, and sliced peaches packed in sirup showed few differences in vitamin content, appearance, flavor, or texture whether frozen very slowly, very rapidly, or at intermediate rates. The retention of these quality factors during a six-month storage period at 0° F. was also unaffected by the freezing rate.

Photomicrographs of sections of the frozen products showed insignificant differences also regardless of the rate of freezing.

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Studies on the Rate of "In Vitro" Pancreatic Hydrolysis of Protein[a]

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A study has been made of the rate of hydrolysis by pancreatin of spray-dried egg white and ration bars containing it, as affected by various experimental conditions. Advantages are shown of reporting results by use of the "digestibility index" which is independent, within wide limits, of the protein content of sample used, or of the duration of the experiment.

Introduction

Melnick, Oser, and Weiss *(3)* have emphasized the importance of determining the rate of in vitro enzymatic hydrolysis as an index of change in nutritional value of a given protein undergoing heat processing. They followed the course of the reaction by determining the degree of hydrolysis after 1, 2, and 5 day intervals employing samples whose weights were selected to contain initially a constant quantity of protein calculated from Kjeldahl analysis. The results were expressed as percent hydrolysis for the three different times of digestion, and comparisons of the raw and treated protein were made on this basis.

In the course of a study of the effect of storage on digestibility[b] of protein contained in experimental ration items, it was not found feasible to keep the protein content of the sample constant due to the limited amounts of material available. The difficulty was overcome by investigating the mathematical relationship between percent protein hydrolyzed, the initial content of protein and the time of hydrolysis, enzyme concentration being kept constant. It was found that in the protein substances investigated the percent digested was proportional to the square root of the initial protein content, and to the time *(6, 7)*. The constant so obtained is characteristic of a given protein preparation regardless of protein content of sample or of digestion periods used (1-5 days). Thus the digestion technique becomes simplified in that specified amounts of protein or periods of time are not essential for interpretation or comparison of results and the choice of these variables may become a matter for the convenience of the operator. The usefulness of this method of expressing results of in vitro digestibility studies is demonstrated in the work reported below.

[a] This paper reports research undertaken by the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 202 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

[b] While the word digestibility is ambiguous, it is used here as equivalent to the more awkward but more exact "rate of hydrolysis."

Experimental Procedures

A study was made of the effect of heat processing on commercially spray-dried egg white and on egg white contained in low protein vehicles in the form of emergency ration bars.[c] Rates of digestibility were determined by the in vitro procedure of Melnick, Oser and Weiss *(3, 4)* with minor modifications due to the special problems presented by the physical properties of the experimental material and by the different protein content of samples. The effects of autoclaving egg white at 15 pounds pressure for 10 and 40 minutes and steaming at 100° C. for 10-180 minutes were investigated. Prior to the heat treatment, 2, 6, or 10 grams of dried egg white were suspended in distilled water to a volume of 90 ml., care being taken to ensure thorough wetting of the powder. The mixture was well whipped in a Waring Blendor, transferred to a 250 ml. wide-mouthed glass-stoppered bottle and then subjected to the heat treatment described above. After cooling, 10 ml. of toluene and 10 ml. of a suspension of 100 mg. U. S. P. pancreatin in $\frac{M}{5}$ phosphate buffer, (pH 8.5), were added. The total mixture (110 ml.) was put briefly through the Waring Blendor, and a 10 ml. aliquot taken for formol titration ("zero time"). The remaining mixture was adjusted to pH 8-9 as indicated by test paper and stored in a tightly stoppered bottle at 37.5° C. Aliquots were then taken for titration at 24, 48, and 120 hours. The pH was kept between 8-9 throughout the course of the digestion by the addition of 10N alkali. Enzyme blanks were run for each series of experiments.

Rate of digestibility was also determined on 10 gram samples of the dried, ground, defatted ration bars, using the same technique of hydrolysis and titration.

The digestibility data were calculated in three different ways:

a. Increase in formol titration per 10 ml. aliquot, in ml. 0.0896 N alkali

[c] Five batches of modified cookie dough containing 60% of the protein as egg white and varied only to produce flavor and texture differences were baked in a 400° F. oven for 15 minutes (average internal temperature 120° C. for 10 minutes) and then ground and compressed into the 5 types of bars as indicated in Table 3. The protein content of these bars ranged from 23 to 27%. Other ration bars not reported here contained as little as 13 to 16% protein.

b. Percent hydrolysis [4]

$$= \frac{\text{increase in formol titration} \times 100}{\text{calc. maximum increase in formol titration}}$$

$$= \frac{\text{increase in formol titration} \times 100}{0.1015 \times \% \text{ protein} \times \text{gm. sample}}$$

c. Percent hydrolysis per gram½ protein per day½

$$= \frac{\text{increase in formol titration} \times 100}{1.015 \times \sqrt{\text{days}} \times \text{gm. sample} \times \% \text{ protein}}$$

Results

The data in Table 1 show the effect of varying sample size on the 1, 2, and 5 day digestibility of egg white autoclaved 10 and 40 minutes. It may be seen that although the actual titration value increases with size of sample and time of digestion, and the percent hydrolysis decreases with increasing amounts of sample, the digestibility index or the percent hydrolysis per gram½ protein per day½, remains substantially constant at a given heating time independently both of sample size and time of digestion. However, the sample heated for 40 minutes has twice the digestibility of that heated 10 minutes (28 instead of 14%). It is apparent that the trypsin inhibitor activity of egg white survived the spray drying process.

TABLE 1

Factors Affecting the Digestibility of Autoclaved Egg White

Weight of Sample	40 minutes at 120° C.		
	Days		
	1	2	5
	(a) ml. increase in formol titration (0.0896 N alkali)		
2	3.1	4.8	6.6
6	5.7	7.7	13.6
10	7.3	13.0	21.1
	(b) percent digestion		
2	22	33	46
6	13	18	31
10	10	18	29
	(c) percent digestion per gram½ protein per day½		
2	26	28	25
6	27	26	29
10	27	34	35
	10 minutes at 120° C.[1]		
10	a) 33	5.1	8.3
	b) 5	7	12
	c) 12	14	14

[1] This included 5 minutes at full pressure and 15-20 minutes cooling time in the autoclave. It was found that by this procedure it was possible to maintain a temperature of approximately 120° C. for 10 minutes. (1 mg. pancreatin/ml. 37° C.).

TABLE 2

Effect of Heating Time on Digestibility of Dried Egg White Held at 100° C.[1]

Minutes at 100 C.	Day	Increase Formol Titration (a)	Percent Hydrolysis (b)	Percent Hydrolysis Per gm.½ Protein Per Day ½ (c)
0	1	0.4	0.5	1.5
	2	0.9	1.2	2.3
	5	2.0	2.7	3.3
Average				2
10	1	2.2	3.0	8.0
	2	3.6	4.9	9.3
	5	4.4	6.0	7.2
Average				8
40	1	4.0	5.4	14.6
	2	6.4	8.7	16.6
	5	9.7	13.2	15.8
Average				16
100	1	5.0	6.8	18.3
	2	8.4	11.4	21.7
	5	14.5	19.7	23.7
Average				22
180	1	4.4	6.0	16.1
	2	6.6	9.0	17.0
	5	11.8	16.0	19.3
Average				18

[1] 10 gram sample (1 mg. pancreatin/ml. 37° C.).

The data in Table 2 show the effect of varying heating time at 100° C. on the digestibility index. It is seen that the percent hydrolysis per gram½ per day½ remains constant (within experimental error) for a particular heating time; it increases with time of heating from 2% for unheated egg white to a maximum of 22% for egg white heated 100 minutes and then decreases again on further heating up to 180 minutes.

These data suggest that the effect of heat on the digestibility of egg white protein is a resultant of at least two separate reactions: (a) the destruction of the trypsin inhibitor which results in increased digestibility *(2)* and (b) the alteration in the structure of the other proteins of egg white which results in decreased digestibility. It is apparent that under these experimental conditions the opposing effects of the two reactions reach a balance upon exposure to the 100° C. temperature for 100 minutes as represented by the optimum digestibility obtained under these conditions. (See Table 2).

The effect of heat on the increase and subsequent decrease in digestibility of egg white as shown by this technique does not necessarily reflect parallel changes in the biological availability of this protein. In this connection, Klose et al *(1)* have shown that the trypsin inhibitor from egg white has no detrimental effect on the growth of rats comparable to that shown by similar inhibitors from soybean and lima beans. This emphasizes the fact that the in vitro digestion technique is an empirical one and must be correlated with biological evaluation for each protein individually.

The data in Table 3 show the percent hydrolysis per gram½ protein per day½ for egg white-containing cereal bars, stored under various conditions. It may be seen that the digestibility index of the protein in the bar does not show any consistent trend on varying storage conditions or the type of bar (average 14%). The differences that are shown are probably to be ascribed

[4] It was assumed, in calculating maximum increase in formol titration, that 7.5% of the nitrogen of intact protein, and 95.0% of the nitrogen of hydrolyzed protein were formol titratable (4, 5). Thus the calculated maximum increase in formol titration

$$= \frac{10 \times \% \text{ prot.} \times \text{gm. sample} \times 1000 \times (95.0\text{-}7.5)}{110 \times 100 \times 14 \times 6.25 \times 0.0896 \times 100}$$

or $= 0.1015 \times \%$ protein $\times$ gm. sample. Hence maximum increase in formol titration per gm. protein

$$= 0.1015 \times 100 \times 1 = 10.15$$

TABLE 3

Percent Hydrolysis per gram$^{1/2}$ Protein per day$^{1/2}$ in Stored Cereal Bars[1]

Bar	Conditions of Storage						
	Days	Unstored	—21° F.		100° F. (air)		
			1 mo. air	1 mo. CO_2	1 mo.	2½ mo.	4 mo.
	Digested						
Oatmeal	1	11	16	15	16	12	17
	2	12	15	17	19	15	16
	5	14	15	18	19	19	16
Cheese	1	13	14	12	10	10	19
	2	11	15	15	12	10	14
	5	16	15	15	14	14	14
Chocolate Malted Milk	1	10	16	11	10	9	9
	2	12	13	13	11	11	10
	5	20	13	15	14	14	11
Vanilla	1	6	8	14	18	15	6
	2	12	12	16	21	18	12
	5	18	11	17	21	21	13
Cocoanut	1	12	11	9	10	10	5
	2	13	13	12	13	12	6
	5	11	12	14	15	14	10

[1] The cereal bars contained approximately 60% of their protein as egg white.

to the heterogeneous nature of the bars and the fact that small samples available did not permit replication. It was interesting to note that the digestibilities of these bars were approximately equal to that of egg white receiving a heat treatment similar to that of the dough from which the bars were made (120° C. for 10 minutes. See Table 1 and note 2.) These data are given for illustrative purposes only; they form a small part of a much more extended series of experiments, all of which show similar results. As these bars were made in a different division, no part of the process of production or storage was under the authors' control. The random variation even with the same type of bar is illustrated by the use of three controls—the unstored bars, those kept 1 month at —21° F. under air, and those kept at —21° F. under CO_2.

Acknowledgment

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A Procedure for the Quantitative Estimation of Salmonella in Dried Egg Products[a]

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A proposal is made of a procedure for the determination of the degree of contamination of dried egg products by members of the genus Salmonella.

Evidence adduced by British, Swedish and American investigators has been held to suggest strongly that bacteria belonging to the genus *Salmonella* in dried egg products are responsible for certain outbreaks of food poisoning. However, the evidence is circumstantial and somewhat inconclusive. There is no question but members of this group have been isolated repeatedly from egg products. The presence of *Salmonella* in whole egg powder has been regarded as a potential health hazard. Dried yolk and dried albumen have not received such serious scrutiny. The question of the presence or absence of these bacteria in dried albumen is of special interest inasmuch as a spontaneous fermentation of the liquid egg white prior to drying gives opportunity during the conditioning period of two to seven days for marked changes in the bacterial flora. Gibbons and Moore *(4)* concluded whole egg powder contained less than one organism of this type per gram, while Ayres and Slosberg *(2)* recorded much higher numbers in dried albumen.

Members of this group show considerable longevity in dried egg products as attested by the results of Wilson *(12)* who worked with dried whole eggs, Gibbons and Moore *(5)* with similar material held at ordinary temperatures of storage, and of Ayres and Slosberg *(2)* who employed elevated storage temperatures and found that these bacteria in dried albumen died off more slowly than reported in dried whole egg.

The processor who wishes to manufacture a low count product is placed in a difficult position because of the uncertainties in isolating and identifying members of the genus *Salmonella*. The techniques proposed, involving a series of trials culminating in biochemical and agglutination tests, are so cumbersome that they dis-

[a] Journal Paper No. J. 1602 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 970.

courage attempts at adequate analyses. For example, Gibbons and Moore *(4)* reported a method for estimating the survival of a seeded type of *Salmonella* in samples in which these organisms were detected by various morphological, biochemical and agglutination tests. After securing this qualitative information, a quantitative study was made by use of three decimal series of ten culture tubes, to each being added 1.0, 0.1, and 0.01 gram of whole egg powder ground in a mortar with sand and tetrathionate broth. Following incubation of the tubes, positive samples were determined by streaking the broth on SS agar. The colorless colonies were checked as to type by use of 0-antisera. Later Gibbons *(6)* reported the most probable number (M.P.N.) of *Salmonella* contaminants in unseeded commercial spray-dried whole egg powder to be less than one organism per gram.

Processor and sanitarian alike are interested in quantitative rather than qualitative methods for determining *Salmonella* largely because they would aid: (1) in tracing the source of contamination, (2) in determining the extent of contamination of their products from time to time and, (3) in obtaining information on the changes in numbers of these microorganisms during processing and storage. In eggs, as in a number of food products wherein bacteria of this group are present, a comparatively simple method of testing is needed that will yield quantitative data. Ordinarily, the number of viable pathogens in the dried material has been found to be so small that customary plating procedures are of but limited value.

The method described herein is purely quantitative. It makes use of some of the procedures proposed by Gibbons and Moore but no attempt is made to utilize tests primarily designed to determine the individual serotypes of *Salmonella* present. No use is made therefore of polyvalent 0-antisera. The method is directed primarily at a determination of the extent of the contamination.

Materials and Methods

Representative samples of dried whole egg, yolk, and albumen were obtained from a large commercial egg breaking and drying plant. Total and coliform counts were obtained at the plant at the time of sampling. The egg products were then shipped the same day via air express to the station bacteriological laboratory and for the most part were received the next day.

The samples were analyzed for *Salmonella*. One gram amounts were placed with 9 ml. of sterile water or physiological saline. The sample remained at room temperature for two hours to permit reconstitution when dilutions were made. Then 1 ml. and 0.1 ml. inocula were spread on SS agar (Difco) and on Kauffman's [b] Brilliant Green agar (KBG agar) plates. The mixture was evenly distributed over the surface of the media by use of a sterile platinum wire. Before inoculation the plates were dried for several hours to evaporate the water of syneresis. All plates were incubated for 24 hours at 37° C.

[b] Kauffman's modification *(9)* of Kristensen, Lester and Jurgens' *(10)* Brilliant Green agar.

On SS agar surfaces, small, smooth translucent colonies were presumed to be representative of the *Salmonella* group. On KBG agar *Salmonella* colonies produce an alkaline reaction, are pinkish-white, opalescent, and surrounded by an intensely red area.

Transfers from the centers of typical colonies were stabbed into, and streaked upon slants of Krumwiede Triple Sugar agar or Triple Sugar Iron agar; if, after 18-20 hours, the butt of the tube showed acid (yellow) and gas (bubbles) while the surface of the slant remained unchanged (red), a microscopic examination of Gram-stained material was made to determine whether or not the organisms were Gram-negative, short, nonspore-forming rods characteristic of organisms belonging to the genus *Salmonella*. When confirmation was desired, tubes of urea broth and SIM medium (Difco) were inoculated from the slant.

For samples showing negative results in 1 ml. dilutions (0.1 g. dried egg product) the following procedure was also used: after thorough mixing, decimal amounts of dried egg (ranging from 0.1 g. to 100 g.) were weighed aseptically on sterile paper. The portions then were added to Selenite-F or tetrathionate enrichment broths. When 10 or 100 g. amounts of dried egg product are being tested it is desirable to add these to Erlenmeyer flasks containing respectively 90 and 500 ml. quantities of enrichment broth. The inhibitory action of these media upon coliforms is of considerable importance when testing dried fermented albumen in order to eliminate the large numbers of *Aerobacter* and *Escherichia* associated with spontaneous fermentation of that product.

The 0.1 g. samples were placed in 3 ml. of the enrichment broth in 13 x 120 mm. tubes while the 1 g. portions were added to 9 ml. of Selenite-F in 18 x 150 mm. tubes.

After incubation at 37° C. for 18-20 hours, each dilution was streaked on SS agar or KBG agar and, after growth on subculture, typical colonies were confirmed by the procedure described previously.

Most probable numbers of *Salmonella* were obtained by testing known weights of dried egg product in replicate and comparing the numbers of *Salmonella* positive in each dilution with tables of most probable numbers prepared by McCrady *(11)*, or Hoskins *(8)*.

Results

The early trials were made using dried egg albumen. It was found that the numbers of *Salmonella* were so low as to make it very difficult to obtain counts using selective media and ordinary plating methods. Direct addition of the albumen to SS agar or to KBG agar resulted in the formation of an opaque precipitate that interfered with plate reading. Also, with the large amounts of sample required in the case of reconstituted dried whole egg or yolk, excess liquid remained on the surface of the plate throughout the incubation period; this prevented development of discrete colonies. Also, there were always so many coliforms present in commercially dried egg products that they interfered with the determination of *Salmonella*. For these reasons, it was considered desirable to subculture decimal quanti-

ties in an enrichment medium which would permit any viable *Salmonella* cells to reproduce enormously and at the same time inhibit growth of coliforms. If any members of the genus *Salmonella* were present, it was assumed that their numbers would increase so that it would be possible to isolate them by transferring one or two loopfuls of the enrichment broth to SS agar.

Lack of turbidity in the higher dilution tubes of Selenite-F gave a rough indication of the amount of contamination in the product. This could be used as a check on subculture tests but was not sufficiently reliable when used alone. Growth of coliforms, paracolons, *Proteus, Bacillus, Pseudomonas* and other bacteria was observed to be responsible for part or all of the turbidity in various tests. Good correlation was obtained between clear Selenite-F tubes and negative subcultures; however, the presence of egg product clouded the liquid when more than 0.01 g. was present in the enrichment broth tube.

Subcultures from single tubes of enrichment broth inoculated in geometric series with 10, 1, 0.1, and 0.01 grams of dried egg albumen showed only small numbers of viable *Salmonella*. While such diluting to the point beyond which no growth occurs is commonly used to give an indication of the incidence of various organisms in food products, it has but limited value since the results obtained are only very roughly quantitative.

Table 1 shows that if a procedure analogous to that described in Standard Methods for the Examination of

TABLE 1

Results Secured in Determining Numbers of Salmonella in Commercial Dried Albumen

100 g.[c]	10 g.	1 g.	0.1 g.	0.01 g.	Indicated Number per gram	Indicated Number per 100 grams
+[d]	+	−[d]	−	−	0.1	10
+	+	+	−	−	1.0	100
+	+	+	+	−	10.0	1000
+	−	+	−	−	0.1	10
Arithmetic average					2.8 g.	280
Number of Tubes Positive					Most Probable Number per gram	Most Probable Number per 100 grams
4	3	3	1	0	0.3	30

[c] Grams of dried albumen added to Selenite-F. After being in Selenite-F enrichment for 18-20 hours, material was subcultured to SS agar and if a typical colony confirmed on Krumwiede's Triple Sugar agar, a positive presumptive test for *Salmonella* was given.

[d] + = positive presumptive test; − = presumptive test negative.

Water and Sewage *(1)* for the approximation of coliform densities is used for estimating *Salmonella* contamination in dried egg albumen, entirely different "indices" are obtained for each of the several replicates. Also, as may be seen in Table 1, when the estimate of *Salmonella* population based on positives from single portion decimal dilution tubes is adopted, the indicated number (I. N.) of *Salmonella* for trial No. 1 is 10 *Salmonella* per hundred grams, whereas for trials No. 2 and No. 3 the same sample yielded values of 100 and 1,000 respectively. Obviously, such method of sampling gives but a rough estimate when used quantitatively. This is particularly true when comparatively few tubes are used.

However, since all of the individual trials were replicate decimal plantings of a single sample, it was possible to make an estimate of most probable numbers using the probability tables of McCrady *(11)*. By this means an M. P. N. of 30 *Salmonella* per 100 grams was obtained. This value contrasts with the average number of organisms per gram determined by adding the I. N.'s in the four trials (280 per 100 g.). Due to the large number of bacteria in dried egg products, it was necessary to use a selective medium that would enrich and differentiate *Salmonella* organisms from the remaining flora.

In early trials SS agar was employed as the selective medium. However, growth of *Proteus* or paracolons produce colorless or black colonies which may be confused with *Salmonella*. Use of SS agar for quantitative isolation of *Salmonella* may be questioned unless study of each colony is made to rule out "aberrant coliforms" and *Pseudomonas* and *Proteus*.

KBG agar was less inhibitory to coliforms than SS agar and, consequently, direct platings had to be made at sufficiently high dilution that the coliforms did not completely overgrow the plate. While these organisms gave an entirely different color reaction than did the *Salmonella*, they crowded the latter organisms so that there were areas where the organisms coalesced and were difficult to determine with certainty.

A larger number of positive tests for *Salmonella* on transfer from Selenite-F enrichment broth to KBG agar were yielded than was the case when SS agar was used. Further, total *Salmonella* counts on direct transfer were higher in KBG agar than in SS agar. While subcultures of some of the turbid enrichment broths indicated more profuse growth of coliforms or other atypical colonies than was the case in SS agar, picking of colonies for transfer to differential tube media such as Krumwiede's Triple Sugar or TSI agars was reduced to a minimum since slow lactose and sucrose fermenters did not produce forms readily mistaken for typical *Salmonella* colonies. According to Broh-Kahn *(3)* growth at the end of twenty-four hours on KBG agar is almost certain to be due to *Salmonella;* presence or absence of growth on this medium at that time "constitutes the most reliable single differential test for the presence of *Salmonella* organisms."

Many of the difficulties experienced in distinguishing *Salmonella* from *Pseudomonas, Proteus* and paracolons in SS agar were greatly reduced when KBG agar was employed. Cultures of *Proteus vulgaris* and *P. morganii* and of various paracolons were inhibited; those that were not completely suppressed appeared greenish-white on a green background, making them easy to distinguish from the typical pinkish-white *Salmonella* surrounded by an intense red background.

Some species of *Pseudomonas* gave a red background. For the most part the colonies were slightly discolored, peaked and with rough edges. However, transfer to differential tube media was necessary to rule out possibility of the organisms in question being members of the genus *Pseudomonas*. Colonies of species of *Bacillus* produced a red background on KBG agar but the colony appearance was easy to distinguish. Organisms of the

genus *Bacillus* produced large, spreading, flat, rough, irregular, opaque colonies while *Salmonella* colonies were smaller, round, raised and opalescent.

Results obtained by the direct transfer of reconstituted egg products and by the use of M.P.N. data from Selenite-F enrichment followed by streaking on SS agar are shown in Table 2.

TABLE 2

Comparison of the Enrichment Dilution and Direct Plating Methods for Determining the Presence of Salmonella in Commercial Dried Egg Products

Type of Product Analyzed	Numbers of Salmonella Determined by:			
	Enrichment and Subculture in SS agar [e]		Direct Plating [f]	
	A	*B*	*C*	*D*
Dried Egg Albumen	100 g.	.1 g.	.1 g.	.01 g.
Lot No. 1	2,000	2	9 ppt.[g]	0
Lot No. 2	700	.7	0	0
Lot No. 3	540,000	540	300	83
Dried Egg Yolk			clouding	
Lot No. 4	210	.21	1	0
Lot No. 5	240	24	0	0
Dried Whole Egg				
Lot No. 6	.45	.00045	0	0
Lot No. 7	.62	.00062	0	0

[e] Most Probable Numbers value (A) per 100 g. of dried egg product (equivalent to 1,000 ml. of reconstituted egg product); (B) per .1 g. of dried material.

[f] Dried material reconstituted 1:10 (i.e., .1 g. material to give total volume of 1 ml.).

[g] Plate counts were limited by ability to spread material over surface.

No valid counts were obtained by direct plating of dried whole egg or yolk, and only in the case of grossly contaminated dried albumen was it possible to determine quantitatively by this means the number of *Salmonella* organisms surviving in the dehydrated material. Subculture of one ml. of reconstituted egg produce caused the plates to become so opaque that accurate reading was very difficult. Also, unless quite dry, it was not possible to spread more than 0.2 ml. reconstituted egg dilutions over the agar surface with any assurance that it would not "run."

Table 3 shows that the numbers of colonies usually were so small that they did not constitute valid counts by the usual criterion (30 to 300 colonies per plate). For example, in sample No. 3 of reconstituted albumen using the lowest dilution possible without clouding or formation of excessive precipitate, i.e., 0.1 g. of the dried material or 1 ml. of reconstituted product, direct plate counts of 11, 0, and 4 colonies were obtained. However, when plates containing typical *Salmonella* were considered positive and data from the replicate samples used collectively to determine most probable numbers, an indication of the amount of contamination was obtained. While two of the three plates in the lowest decimal plantings gave positive presumptive tests for *Salmonella*, only one of the next series of triplicate plates and none of the three highest dilution plantings indicated *Salmonella*. Therefore, the number of positive findings, planting three portions in each of three dilutions in geometric series, were 2/3, 1/3, and 0/3. Comparison of the results from these dilutions with McCrady's tables indicated an M.P.N. of 15 *Salmonella* per gram. This agreed surprisingly well with the M.P.N. of 14 *Salmonella* per gram obtained by use of the enrichment method described above. Other M.P.N. values in this series also were in very good agreement. In the direct plating method the most probable numbers technic may be applied to enlarge the range over which direct plating has been used quantitatively.

Sample No. 4 (Table 3) illustrates the alternate possibility of getting a direct plate count when the numbers of *Salmonella* are unusually high. While the load of these microorganisms in this particular sample is exceptionally high, the direct count does illustrate that the overall area covered by use of the plating method in both ways, direct and by M.P.N.'s, is increased for both the high and the low dilutions.

Discussion

Usually the study of suspected pathogenic bacteria in food products is facilitated by adding unit quantities of the material to a liquefied solid medium. For this purpose, at best, such direct plating technics are subject to many errors. Clumping of cells, inhibitory effects of organisms upon each other, toxicity of the substrate,

TABLE 3

Numbers of Salmonella in Commercial Dried Fermented Albumen as Determined by Direct Plate Count, by Decimal Dilutions on Plates and by Multiple Tube Dilutions in Enrichment Broth

Sample No.	Plate Count					Multiple Tube Dilution Count (5 tubes at each dil'n)					
	0.1 g.	.01 g.	.001 g.	Av. by direct count per gram	M. P. N./g.[i]	0.1 g.	.01 g.	.001 g.	.0001 g.	.00001 g.	M. P. N./g.[i]
1 a)	32 [h]	5	0								
b)	25	1	0	24		5	4	2			220
c)	16	0	0								
Positive plates	3	2	0		95						
2 a)	17	0	0								
b)	9	0	0	11		4	3	1			23
c)	7	0	0								
Positive plates	3	0	0		25						
3 a)	11	3	0								
b)	0	0	0	5		3	1	1			14
c)	4	0	0								
Positive plates	2	1	0		15						
4 a)	> 300	51	6								
b)	> 300	83	11	6700		5	5	5	3	0	7900
c)	> 300	66	9								
Positive plates	3	3	3		1400 +						

[h] Colonies presumed to be *Salmonella* as determined by macroscopic appearance on KBG agar; representative colonies confirmed on T. S. I. agar.

[i] MPN of *Salmonella* determined by comparison of positives in significant dilutions with McCrady's tables.

and overcrowding of colonies on the plate must always be considered. Especially important are the errors of dilution and distribution. In low count samples the distribution error alone may reduce quantitative accuracy to a very low level. For this reason plates containing less than 30 colonies usually are rejected. On the other hand, the number of *Salmonella* in dried egg products is generally so small that dilution of the material to a point that will eliminate clouding or formation of precipitate results in low count plates.

By applying "most probable numbers" tables to positive but low count samples, a useful approximation can be had regarding counts. Also, if positive readings of triplicate(or more) plates from the several dilutions tested are compared with M. P. N. tables, the above procedure will increase the area of usefulness of plate counts.

Nevertheless, there are many products of the dried egg industry that cannot be measured by the direct plate count. The multiple dilution method, whereby any amount of product can be sampled, is clearly superior to the plating technic for this particular use since its limitations are governed only by the cost of materials and time involved in making the test. With the latter procedure the number of replicates determine the precision expected.

Halvorson and Ziegler *(7)* have shown that, if less than twenty tubes are used with each dilution, the errors of sampling are greater than when twenty or more tubes are used. For routine testing methods the use of twenty tubes is impractical. Results secured with smaller numbers of dilutions are sufficiently accurate for all practical purposes, and for this reason triplicate plate samples at each dilution are used for plate and range-finding counts and quintuplet tubings at decimal dilutions are employed for determining M.P.N.'s in this laboratory.

Conclusions

The following procedure has been developed for determining the degree of *Salmonella* contamination in dried egg products.

The dried material is weighed aseptically in 0.1, 1, and 10 gram portions and these added to tubes or flasks containing 3, 9, and 90 ml. of Selenite-F enrichment broth respectively. Larger or smaller portions can be tested but, when smaller samplings are made, the dry egg product is reconstituted and suitable dilutions added to the enrichment medium. It is recommended that five tubes be used for each of the serial dilutions although when tests are made in the field or when conservation of materials is required, it is possible to use fewer replicates. After the tubes have incubated at 37° C. for 16-20 hours, a loopful of the various dilutions of egg product in enrichment broth is streaked on KBG agar and the plates incubated at 37° C. for 24 hours. On the KBG agar plates the presence of pinkish-white opalescent colonies surrounded by a bright red area is regarded as a presumptive test for the presence of *Salmonella*. If the colonies produce typical *Salmonella* reactions, Most Probable Numbers data then may be obtained by comparing the number of positive plates at each dilution with the corresponding number in probability tables computed for the same number of replicates. Typical colonies are picked from the plates and transferred to Krumwiede's Triple Sugar or to TSI agar tubes. Assuming that each viable *Salmonella* organism increases in numbers in the enrichment broth, the figures make it possible to use Most Probable Numbers tables for estimating the degree of contamination.

Although this is as far as the worker ordinarily would proceed, confirmation and even completion tests can be carried out in the orthodox manner by running urease and SIM tests, fermentation reactions, and agglutination tests.

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Notes and Letters

"Modesty Forbids"

In a recent exchange of correspondence with The Editor certain material came to light which seems very appropriate for this section of the Journal. The material referred to was in the form of letters commenting directly to The Editor regarding the new format of the Journal, some of which follows:

* * * *

"I have in my hands one of the new issues of FOOD TECHNOLOGY and I want to say that I think you are to be congratulated on the splendid appearance and format of the new Journal.

"I feel that this is a publication worthy of the organization for which you and many of us labored so hard—I am thinking now about the I. F. T. in general—and that the Journal is a tribute to you and the tremendous amount of work and effort you have put in so efficiently in making it a journal of which we can all be proud. I don't think many people realize the tremendous amount of time and effort you have put into this endeavor and how efficiently you have done it despite numerous and sometimes difficult obstacles. I want you to know, however, that I think I do appreciate the circumstances and I feel that every I. F. T. member owes you a sincere debt for making such a publication possible.

"I hope you will continue to carry the leadership of this journal for a long time to come.

"With congratulations and best regards."

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"The January 1949 issue of FOOD TECHNOLOGY has just reached me and I want to extend to you my hearty and sincere congratulations for a job well done. The journal is excellent. It is attractive in appearance and makeup, it is well printed, well executed and it is a credit to our organization. The maiden issue and the new format normally would have been incomplete in many details but such is not the case here, as this issue represents a highly acceptable and finished product.

"I have one minor suggestion to make which I believe would be of use to all readers of the journal and that is the inclusion of the address of the authors whose articles are abstracted in the Selected Abstracts. The American Chemical Society has finally gone over to giving the addresses in the Chemical Abstracts and they are very helpful. If we could have them in this journal, I think it would be of help to all of us likewise.

"Kindest regards."

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"FOOD TECHNOLOGY is being received promptly and continues to be of such character that we look forward to its coming. You are to be commended on the fine job that you are doing with this journal. I am quite concerned, however, and thus the reason for writing you at this time, that volume 3 is appearing in an entirely different size than volumes 1 and 2. Promptly upon receipt of the last number of the volume I have the entire volume bound. The new size does not permit the same size binding as did volumes 1 and 2.

"I am wondering if the size now being used is temporary or will that be the size of *FOOD TECHNOLOGY* in the future? I appreciate there were reasons for changing the size of the journal but it does not lend itself to consistency in the bound volumes."

EDITOR: "The change in size is a permanent change and was made when it was decided to accept advertising because advertising plates are commonly made to fit our new size. We regret the necessity for changing the format but the step was taken as a measure to increase the income of the Journal. You will find a discussion of the matter on the second page of the issue of July, 1948, and on the inside back cover of the issue of January, 1949."

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"May I add my sincere congratulations to the many others you must have received for the 'swell' issue of FOOD TECHNOLOGY, which reached my desk just the other day.

"You and your staff can well feel proud of this initial effort in the new format."

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These are abstracted for your appraisal. Do you agree with these views? Do you have further suggestions regarding the Journal? Do you agree with this writer that The Editor has been very modest in withholding this appreciation of his accomplishments?

NEW YORK CITY FOOD STATISTICS

It is estimated by the New York City Department of Health that the 8,067,000 persons in New York City each consumes 4.6 pounds of food and liquids (including milk) a day or a total of 37,108,200 pounds for the City as a whole. New Yorkers also use huge quantities of drugs, cosmetics and other like preparations every 24 hours.

It is the responsibility of the Bureau of Food and Drugs of the Department of Health to see that this food supply is wholesome, that the more than 100,000 establishments through which that food flows to the consumer meet sanitary standards and that all drugs are pure and that cosmetics and similar preparations are not harmful.

In the first eleven months of 1948 Food and Drug Bureau inspectors made 199,721 inspections; investigated 5,166 complaints by citizens, firms and agencies; took 28,510 samples for chemical and bacteriological analysis; condemned 1,365,837 pounds of food as unfit for use and took 96,390 pounds of unsafe and impure drugs, cosmetics and other preparations off the market.

Inspectors served 2,361 summonses charging violations of the Sanitary Code and obtained 2,190 convictions, 60 cases were dismissed in court and 111 cases were still pending on December 1st. The fines averaged $43.30 for a total of $94,833.